AN EXAMININATION OF BROILER BREEDER BODY COMPOSITION METHODOLOGY AND ENERGY METABOLISM

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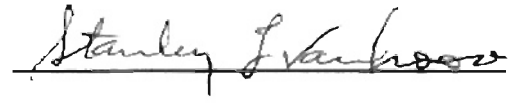

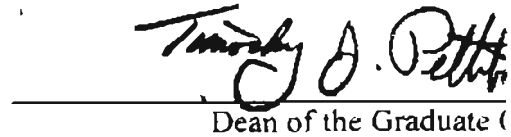

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NOMENCLATURE

AT	ambient temperature
BC	body composition
BMR	basal metabolic rate
BW, BWT	body weight
CNB	carbon-nitrogen balance
CNTC	carbon-nitrogen tissue content
DEXA	dual-energy x-ray absorptiometry
HP	heat production
MBS	metabolic body size
PA	proximate analysis
TN	thermoneutral
U/S	ultrasound

CHAPTER I

INTRODUCTION

Poultry production in the United States has grown rapidly over the last 50 years. In 1950 broiler production was less than 1 billion birds; this number had increased to almost 8.6 billion broilers produced in 2002 with a production value of over $13 billion (NASS, 2003). The supportive section of this industry, broiler breeders, has had to not only grow to meet this numeric demand, but also has had to make genetic, management and nutritional decisions in order to satisfy the changing demands of the consumer.

Body weight and composition are important factors in determining the onset of sexual maturity, as well as reproductive efficiency, in both male and female broiler breeders. Genetic selection has focused on the demand for fast-growing, feed-efficient, heavy-breasted broilers at the production level. However, this has led to reproductive problems at the breeder level that have had to be overcome through nutritional and management programs that control both body weight and composition. Therefore, the challenge to the nutritionist is to formulate diets that meet the metabolic requirements of broiler breeders on controlled feeding programs (i.e. limit-fed).

Determining body composition in broiler breeders is an important consideration to the geneticist in their selection process, to the nutritionist in determining that genetic potentials and metabolic needs are being met, and to the production manager in meeting reproductive goals. Numerous methods have been utilized over the years to determine body composition with proximate analysis serving as the gold standard (Blaxter, 1989). Various morphological measurements and technological methods to measure body

composition have been examined for ease of use, reliability and cost effectiveness. Additionally, being able to determine body composition *in vivo* is of prime concern to the broiler breeder industry.

The studies reported herein were conducted to dynamically examine broiler breeder energy metabolism, expressing energy needs as basal metabolic requirement and metabolic requirement during activity, egg synthesis, and tissue accretion. Further, the relationships between proximate analysis, the gold standard for determining body composition, and selected morphological measurements and technological methods were examined for accuracy, ease of use, and reliability for predicting body composition in broiler breeders.

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CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

Although metabolic and genetic processes dictate the potential for growth and reproductive effectiveness in the broiler breeder, without proper nutritional management neither of these potentials can be met. Nutritional management includes determining the metabolic requirements of the bird and formulating a diet that meets these requirements while controlling body weight (BW) and body composition (BC), both of which have an effect on the subsequent reproductive efficiency of the broiler breeder. Understanding the relationships between these various factors affecting reproductive efficiency provides a firm foundation for making management decisions regarding broiler breeders.

BROILER BREEDER MANAGEMENT

Poultry management in the United States has changed dramatically since the days when people had a backyard flock that provided both meat and eggs for the family's use and a source of income from sales in the local community. During the hard times of the Depression (1929 to 1936) the family backyard chicken flock often provided the economic base for a family's survival (Moreng and Avens, 1985). Management of the backyard flock was simple, consisting of feeding grain and household food scraps to supplement what the birds were able to forage and providing basic housing to protect birds from predators and weather extremes. Genetic controls were very basic; the best

birds, based primarily on appearance, were retained for breeding stock and the inferior birds were culled.

Development of what could be regarded as a commercial poultry industry began in the early 1940's with rapid expansion seen after the end of World War II (Moreng and Avens, 1985). In the beginning this new industry was concentrated in the midwestern states and was still a family-based business with a local support structure (hatchery, feed store, processing facility). By 1960 advances were made in feeding methods and in genetics that resulted in a reduction in market age from 16 weeks to 8 weeks. As the industry grew it migrated into other sections of the United States and changed from being a family-based business to the vertically integrated poultry industry we know today. Scientific and technological advances in genetics, nutrition and management methods have made the growth of the broiler industry one of the wonders of American agriculture.

Development of broiler breeder stock has become a separate industry within the vertical integration of the broiler industry. A substantial amount of scientific research with broiler breeder females has looked at the relationships between reproductive efficiency and numerous factors affecting that efficiency: BW, BC, hormonal influences, food intake, diet composition (protein, energy, amino acids, vitamins, minerals), housing (density, lighting, ventilation, temperature, litter), and bird health (Waldroup *et al*, 1976; Bornstein and Lev, 1982; Bornstein *et al*, 1984; Soller *et al*, 1984; Brake *et al*, 1985; Moreng and Avens, 1985; Robbins *et al*, 1986; Pinchasov and Galili, 1990; Leeson and Summers, 1991; Robinson and Robinson, 1991; Robinson *et al*, 1993; Lewis, 1994; Bartov and Wax, 1998; Decuypere *et al*, 1998). This research has revealed that a negative relationship exists between increased BW and reproductive efficiency.

Therefore, broiler breeder management programs for females all incorporate restricted feed intake with the most severe restrictions seen during the pre-sexual maturity phase (Leeson, 1992). Although less research has been done with broiler breeder males, a similar negative relationship between body size and reproductive efficiency has been identified. As a result, management programs specific for broiler breeder males have been developed (Hocking and Duff, 1989; Wilson, 1990; Leeson and Summers, 1991; Attia *et al*, 1993; Kirby *et al*, 1998).

HEAT PRODUCTION AND METABOLIC BODY SIZE

Heat is produced as a by-product of metabolic processes that occur in the body. Heat production (HP) increases after a meal, during physical activity and in a cold or hot environment. Other major factors that have an effect on HP include age, time of day, and thermal insulation (e.g. hair coat). The contribution of the various organs within the body to HP has also been examined. For example in humans, at rest in a thermoneutral environment, one-half of all HP comes from the gastrointestinal tract, liver and muscle metabolic processes. (Stanier *et al*, 1984)

Maintenance requirement is defined as ". . . that amount of food energy that is needed to balance exactly heat production" (Ruckebusch *et al*, 1991). Beker (1996) used HP data to determine the maintenance requirements of male broilers, ages one week to seven weeks. That study found that maintenance HP per unit BW decreased during weeks one through three then increased during weeks five through seven. Comparison of maintenance HP per unit BW among the different temperature environments revealed that as the environmental temperature decreased the HP per unit BW increased.

A major component of the maintenance requirement of an animal is basal metabolic rate (BMR). Basal metabolic rate is defined as the energy expended by a fasted but not starved animal that is awake but at rest and is in a thermoneutral environment (Bender, 1993). The length of time it takes to reach a fasted state varies among species: humans – 10 to 12 hours, swine - 96 hours, rodents - 10 to 20 hours, ruminants – 3 to 5 days, chickens - 48 hours (Blaxter, 1989).

Basal metabolic rate can be determined through direct calorimetry, which is a measurement of heat loss, and by indirect calorimetry, which determines HP based on gaseous exchange. Several equations have been developed to calculate HP based on oxygen consumption and carbon dioxide production. (Berdanier, 1995). One that is widely accepted is Brouwer's equation (Brouwer, 1965), utilizing oxygen consumption, carbon dioxide production, and excretory nitrogen data, as a basis for determining HP. It was later determined that, since the adjustment generated by the nitrogen data accounted for less than 0.6% of total HP, the excretory nitrogen calculation could be omitted with negligible effect (Romijn and Lokhorst, 1966). This adaptation of Brouwer's equation is as follows:

HP (in kJ/unit time) = [(O_2 consumed in l/unit time)(16.18 kJ/l)] +

[(CO_2 produced in l/unit time)(5.02 kJ/l)]

If needed, the conversion of the HP value to kcal is easily accomplished by dividing the results of the above calculation by 4.184 kJ/kcal.

It has been shown that HP is closely related to heat loss; therefore, since heat loss is a function of surface area, HP is also a function of surface area (Stanier et al, 1984). Body surface area, also known as metabolic body size (MBS), is derived by regressing

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log BMR over log BW with the resultant slope providing an estimate of the exponent used to calculate MBS (Blaxter, 1989). Brody and Proctor (1932) arrived at an estimate of $BW(kg)^{0.75}$ to compare MBS across species. Brody (1945) later determined that $BW(kg)^{0.67}$ more accurately represented MBS in birds.

That a relationship exists between BMR and body size, expressed either as gravimetric BW or as MBS, has been studied for many years. Early work by Brody *et al* (1932) demonstrated a curvilinear relationship between BW in three strains of chickens and BMR/kg BW, wherein BMR/kg BW decreased as total BW increased. Confirmation and refinement of this observation has occurred over time (Kleiber, 1947; Miller, Jr. and Blyth, 1953; Kleiber, 1961; Thorney *et al*, 1976; Heusner, 1985; Johnson and Farrell, 1985).

Since feed is a major cost of production (Moreng and Avens, 1985), identifying the composition and quantity of feed required for basic maintenance and for production is an important consideration. Additionally, environmental effects on the utilization of feed need to be included when determining feeding requirements. Numerous studies have been conducted with broilers to examine the relationship between HP and two or more of the following factors: diet composition, feeding level, and environmental temperature (Farrell and Swain, 1977; Hurwitz *et al*, 1978; Meltzer, 1983; Wiernusz and Teeter, 1993; Jones, 1994; MacLeod, 1997; Koh and MacLeod, 1999). There has also been considerable investigation into the HP of laying hens for both maintenance and egg production (Grimbergen, 1970; Meltzer *et al*, 1982; Li *et* al. 1992). A smaller, although still substantial, body of literature examines factors affecting HP in female broiler breeders. Johnson and Farrell (1983) calculated a maintenance HP value for broiler

breeder hens of 365 kJ/kg $BW^{0.75}$/d. This value is close to, and not statistically different from, the maintenance HP value of 367 kJ/kg $BW^{0.75}$/d calculated by Spratt *et al* (1990a). Higher maintenance HP values for broiler breeder hens, 379 kJ/kg $BW^{0.75}$/d and 534 kJ/kg $BW^{0.75}$/d, were reported in earlier studies by Grossu *et al* (1976) and Balnave *et al* (1978), respectively. These higher values may be due to a difference in the broiler strain and/or changes in environmental and feeding management methods. Heat production attributable to egg production was calculated by Spratt *et al* (1990a) to be 140 kJ/kg BW/d above maintenance levels. The contribution to HP made by different organs and tissues was determined by Balnave *et al* (1978) for the liver and by Spratt *et al* (1990b) for liver, intestines (ileum), reproductive tract (magnum), and muscle (*latissimus dorsi*). Other researchers have examined HP in broiler breeder pullets on food restriction programs, i.e. limit-fed birds (Bennett *et al*, 1990; Pinchasov and Galili, 1990; MacLeod *et al*, 1993). Only limited metabolic research on male broiler breeders is available in the literature. Two studies by Shannon and Brown (1969, 1970) determined maintenance HP in male broiler breeders of 401 kJ/kg $BW^{0.75}$/d and 520 kJ/kg $BW^{0.75}$/d, respectively.

Knowing that both maintenance and production requirements are being met is especially important in limit-fed broiler breeders since deficiencies can result in impaired growth, health and reproduction. Heat production data can serve as a tool for determining these requirements and, therefore, is useful not only to the researcher but to breeder industry in designing their management programs.

BODY COMPOSITION

In the food animal world being able to assess BC is an important concern since lean to fat ratios and size of specific body parts directly relate to the value producers will receive for their product. Over the years various methods have been used to assess BC in animals, however many of these methods are subjective and depend on the experience of the person performing the assessment (Hedrick, 1983). As the laboratory and technological methods available have improved, researchers have had a wider range of methods to select from in order to determine BC for their animal of interest. (Topel and Kauffman, 1988). The gold standard for measuring BC in food animals is, without a doubt, proximate analysis (PA), also referred to as chemical analysis. However since this method requires that the animal be sacrificed, it is not a good tool for selecting breeding stock or for gathering information on changes in BC that occur as the animal grows. Therefore, there is a need for noninvasive methods for assessing BC that allow making accurate measurements *in vivo*.

Morphological Measurement Methods

Over the years external body measurements (e.g. body weight, keel length, head width, pelvis width, abdominal skinfold thickness, shank length and circumference, girth, and breast width, angle and height) have been examined as a method of assessing changes over time in BC, and for estimating body and potential carcass composition in broiler breeders. Instruments have been developed and tested in an attempt to make morphological measurements as objective as possible (Reid *et al*, 1984).

Extensive research has examined the relationship between BW and BC in male broilers (Griffith *et al*, 1978; Summers *et al*, 1988) with the resultant consensus being

that BW is not a good predictor of BC. Robinson *et al* (1996) examined shank length, head width, keel length, and girth in broiler breeder hens that were photostimulated at different ages; no significant differences ($p > 0.05$) were found at trial end (bird age 60 weeks) among the treatment groups for any of the morphological measurements other than for girth. Since girth has historically been used to determine the degree of "fleshing" (i.e. the amount of body muscle, particularly of the breast muscle), it would be expected for girth to be related to differences in body size, as was the case in the referenced study. In this study it was also noted that increased girth was accompanied by a higher fat pad weight, although this relationship was not examined statistically. Latshaw and Bishop (2001) examined several morphological measurements (pelvis width, back length, keel length, breast width, girth, and abdominal skinfold thickness) in five genetic lines, four of which were broiler lines. Models were then developed to estimate BW and BC components based on the morphological measures with accuracy increasing when multiple measurement types were included in the predictive equation.

Market demand in the United States has led the poultry industry to select for increased breast yield; unfortunately this increase in breast size has been accompanied by an increase in fat content (Chambers *et al*, 1981). Although total body fat can be determined accurately ($r^2 = 0.953$) through PA of the bird carcass (Lewis and Perry, 1991), there exists a need to be able to estimate total body fat *in vivo*. Since researchers have shown a moderate to good relationship between abdominal fat weight and total body fat with r^2 ranging from 0.60 to 0.82 (Becker *et al*, 1978; Becker *et al*, 1981; Chambers and Fortin, 1984), the ability to accurately estimate abdominal fat weight would potentially allow for an estimate of total body fat. Using abdominal skinfold thickness,

not including the underlying fat pad, as a predictor of body fat has yielded varying results. While Latshaw and Bishop (2001) found abdominal skinfold thickness combined with BW to be a good estimator of body fat (R^2=0.63), Mirosh *et al* (1980) found a poor correlation (r ranging from –0.03 in male broilers to 0.17 in female broilers) between abdominal skinfold thickness and abdominal fat weight. A caliper methodology, wherein abdominal fat pad thickness was used to estimate abdominal fat weight, was developed by Pym and Thompson (1980). Their calculations showed a moderately good correlation (0.76 for broiler males and 0.75 for broiler females) between estimated abdominal fat weight and actual fat weight. Subsequent studies, utilizing the same basic caliper procedure, have had mixed results with a wide range of correlation coefficient values (r) observed for abdominal fat pad thickness to abdominal fat weight correlations. Gyles *et al* (1982) found very low correlations (r ranging from 0.005 to 0.17, n=260) in a group of mixed-sex broilers. Mirosh and Becker (1984) investigated this relationship and found a very low correlation (r=0.05, n=69) in female broilers and a moderate correlation (r=0.46, n=51) in male broilers. In a group of 904 broilers (sex not stated), Chambers (1982) found low to moderate correlations between abdominal fat weight and abdominal wall thickness measured in two locations (right side, r=0.27; left side, r=0.49); although not stated, inclusion of the fat pad thickness in these abdominal wall measurements is likely. Another moderate correlation (r=0.51, n=36) was observed by Rose and Michie (1983) in a group of mixed sex broilers. The failure of these subsequent investigations to find correlations similar to those found by Pym and Thompson (1980) raises concerns about the usefulness of this technique. One possible explanation for the wide range of values found was raised by Pym (1981) in a study that examined operator effect on abdominal

fat pad thickness data collection. In this study that examined two caliper types, a large difference was observed between the correlation coefficient (relating mean caliper reading and percent abdominal fat) calculated on the data collected by an experienced operator and those data obtained by two inexperienced operators (Table 1). Additionally, Pym (1981) found that the experienced caliper operator's measurements were more consistent, as evidenced by the higher correlation coefficient for repeatability, than those taken by the inexperienced operators. Similar observations were made by Rose and Michie (1983), wherein abdominal fat pad thickness measurements in laying hens by two experienced caliper operators yielded higher correlations to abdominal fat weight per kg BW (r=0.75 and 0.81) than the correlation calculated on an inexperienced operator's data (r=0.59) for the same number of birds (n=36).

Laboratory Methodologies

Proximate analysis, the gold standard for BC determination, is a labor intensive and time consuming process beginning with homogenization of the whole bird or the carcass, depending on the sample unit selected for a particular study. One approach is to freeze then grind the raw sample unit (Chambers *et al*, 1981; Bennett and Leeson, 1990; Lewis and Perry, 1991). Another approach is to heat process the sample unit by pressure cooking (Robinson *et al*, 1996) or autoclaving (McDonald, 1993) before grinding. Extensive sampling and processing to determine dry matter, protein, lipid and ash follows homogenization. Since accuracy is of prime importance, double and triple replicates of each sample are routinely processed. Even with the assistance of automated instrumentation for some of these processes, all of which must meet accepted standards

(AOAC, 1998), these processes are very time consuming for the researcher and their technical staff.

Carbon-nitrogen balance (CNB) has been used historically to determine energy retention during a tissue accretion period. It, like PA, is a labor intensive and time consuming process requiring determination of the carbon (C), nitrogen (N), and energy values of the food entering, the excreta exiting, and any body components (e.g. feathers) shed from the body as well as the amount of C exhaled as CO_2 (Farrell, 1974). Energy retention during the experimental period is then estimated by inserting the retained C and N values, determined by subtraction, into an equation (Brouwer, 1965):

Energy retention (kJ) = (51.83 kJ/g C)(g C retained) – (19.38 kJ/g N)(g N retained)

Additionally, estimates of the change in BC during the experimental period may be estimated from the amount of retained C and N (Blaxter, 1989). These estimates are based on accepted values, originally determined through chemical analysis, of 52.0% and 76.70% for C and of 16.0% and 0% for N in body protein and fat, respectively (Brouwer, 1965). The advantage of using the CNB method is that the animal being studied does not have to be sacrificed, thus allowing the investigation of changes in energy and BC over time in the same animal. However, there is a high potential for introduced error during the many steps involved in both the experimental period (e.g. feed weighing, excreta collection) and in the laboratory analysis period.

Technological Methodologies

Advances in technology have led to more sophisticated methods of determining BC, one of these is ultrasound (U/S). The beef industry has used U/S *in vivo* to ascertain the size of the rib eye and the thickness of backfat in cattle for a number of years

(Brethour 1989, 1990; Henderson-Perry *et al* 1989). Since these two measurements are major components of the equation used by federal graders to determine yield grade in cattle, it is to the producers' advantage to have a noninvasive method of assessing them. Other research has explored using U/S as a tool for predicting future yield in cattle (Smith *et al*, 1989). A study by R□mignon *et al* (2000) using male broilers found that breast muscle area calculated from recorded U/S images was a good predictor of breast muscle mass (R^2=0.84 in one experiment and R^2 =0.78 in another). Bethour (1992) found a high correlation (r=0.975) between consecutive U/S measurements on the same animal when performed by the same operator. The amount of training on interpretation of U/S images was found to be another factor in the determining the reliability of U/S data (McLaren *et al*, 1991). The ability to reliably repeat *in vivo* measurements and to accurately interpret the images increases the potential for using U/S in the field as a tool for determining BC of food animals.

Another technologically sophisticated method is dual-energy X-ray absorptiometry (DEXA) that provides an estimate of total BC (lean, fat and bone mineral). The accuracy of DEXA estimates of BC components in pigs, when compared to chemical analysis, was investigated by Svendsen *et al* (1993). In their study regressions analysis demonstrated strong relationships (r^2 0.98 for lean, 0.99 for fat and 0.93 for bone mineral) between the two methodologies. Dual-energy X-ray absorptiometry was used to measure incremental changes in BC in growing pigs in one study (Mitchell *et al*, 1996) and in growing chickens (Mitchell *et al*, 1997). In both studies regression analysis of BW, as estimated by DEXA, over the actual gravimetric BW determined the two were closely related (r^2=0.99). While the pig study data

indicated that DEXA was a viable method for determining BC changes over time in growing pigs ($P<0.05$), the chicken study results were less conclusive. In that study it was observed that the accuracy of the DEXA measurements varied depending on the weight of the chickens, the body component being measured, and the scanning mode used (Table 2) with the most consistent relationship seen in the $lean_{DEXA}$ to $protein_{PA}$ relationship. In chicks ages 0 to 14 days, Mooney (2000) determined through regression analysis that the best relationship between DEXA estimations and PA determinations of BC was between $protein_{PA}$ and $lean_{DEXA}$ (r^2 0.83), followed by ash_{PA} to bone $mineral_{DEXA}$ (r^2=0.75). The $lipid_{PA}$ to fat_{DEXA} relationship was determined be the poorest (r^2=0.59) among the three. The data from these two studies suggests that further investigation, defining the relationship between DEXA estimates of BC and BC determined by chemical analysis, needs to be conducted in order to ascertain the reliability of DEXA scanning data in predicting BC in chickens.

Other technological methods that have been investigated by poultry researchers include nuclear magnetic resonance imaging and spectroscopy (Mirchell *et al*, 1991) and total body electrical conductivity (Latshaw and Bishop, 2001). Nuclear magnetic resonance imaging and spectroscopy were found to be good estimators of total body water, protein and lipid content ($R^2 \geq 0.93$). Although total body electrical conductivity data, when analyzed alone, proved to not be a good predictor of fat, its inclusion in a predictive equation using other parameters improved that R^2 from 0.63 to 0.78. The usefulness of these and other technologically sophisticated methods of determining BC will continue to be studied. As in the past with older BC determination methodologies,

ease of use, cost, availability, accuracy and safety will all be factors in determining which methodologies will find wide-spread use among researchers and industry.

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Table 1. ***Correlation coefficients (r) generated[1] in a comparison of two caliper designs, used to determine abdominal fat thickness in broilers, used by three operators with differing experience levels***

		Mean[2] caliper reading and Percent abdominal fat (r)		Repeatability of caliper measurement (r)	
Sex (n)	Operator[3]	Caliper 1	Caliper 2	Caliper 1	Caliper 2
Males (16)	1	0.88	0.87	0.81	0.85
	2	0.80	0.82	0.57	0.73
	3	0.55	0.65	0.29	0.17
Females (16)	1	0.78	0.65	0.77	0.73
	2	0.46	0.59	0.42	0.27
	3	0.11	0.08	0.01	0.14

[1] PYM, R. A. E. (1981) Operator effect upon the prediction of abdominal fat in live broilers using a caliper measurement technique *Australian Association of Animal Breeding and Genetics, Proceedings of the 2nd Conference*. pp. 156-157

[2] Of three measurements made with each caliper on each bird by each operator.

[3] Operator 1 was the most experienced operator.

Table 2. ***Relationships determined[1] through linear regression between body composition (BC) components in chickens as estimated by dual-energy x-ray absorptiometry[2] (DEXA) and as calculated with proximate analysis (PA)***

		Relationship (r^2) between BC components (PA/DEXA)		
Bird Size (g)	DEXA mode	Protein/Lean	Lipid/Fat	Ash/Bone mineral
1210 ± 89	Neo-med[3]	0.95	0.42	n/a
2521 ± 63	Neo-med	0.93	0.78	0.05
1205 ± 108	SA-det[4]	0.97	0.65	0.54
2699 ± 111	SA-det	0.95	0.40	0.51
1299 ± 64	SA-det	0.92	0.68	0.57
2458 ± 76	SA-det	0.91	0.81	0.23
1299 ± 64	SA-HR[5]	0.97	0.61	0.63
2458 ± 76	SA-HR	0.90	0.73	0.25

[1] MITCHELL, A. D., ROSEBROUGH, R. W. & CONWAY, J. M. (1997) Body composition analysis of chickens by dual energy x-ray absorptiometry *Poultry Science* 76: 1746-1752.

[2] Lunar™ DPX-L, Lunar Corp., Madison, WI 53700

[3] Neonatal-medium

[4] Small animal-detail

[5] Small animal-high resolution

CHAPTER III

Body Composition and Heat Production of Fed and Fasted Broiler Breeder Females Ranging from 5 to 50 weeks of age During Housing at Three Ambient Temperatures and Oviposition[1]

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SUMMARY

1. Indirect calorimetry was used to measure fed, fasted and basal metabolic rate (BMR) related heat production (HP) of 5 to 50 week old broiler breeder females whose body composition was defined using both dual-energy x-ray absorptiometry (DEXA) and proximate analysis methodologies. Birds were housed at 3 different ambient temperatures (16, 22, or 32 C).
2. Heat production (kJ/bird/h) was decreased ($P<0.05$) within all ages by fasting, whereas overall HP (kJ/bird/h) increased quadratically with bird mass.
3. Regressing log HP of birds held under BMR conditions vs. log body weight yielded a classic exponent of 0.67 for converting live weight to metabolic body size.
4. Body composition changes, tracked by age with DEXA and proximate analysis procedures, yielded results with excellent correlation ($R > .95$) for lean, lipid and bone masses. Equations relating the two methods are presented.
5. Regressing HP during BMR on body composition components (lean, fat and bone mass) determined with DEXA yielded equations relating bird composition and BMR.
6. Data estimate the energy costs of oviposition at 45.85 kJ/bird/day.

INTRODUCTION

Broiler breeder pullet growth rate is normally restricted in an attempt to match body weight (BWT) and sexual maturity for enhanced reproductive performance (Robinson *et al.*, 1993). Breeder management guides typically provide feeding regimens to synchronize bird BWT with age (Cobb-Vantress, Inc., 1998). However, BWT is not

the only factor related to subsequent reproductive performance (Bartov and Wax, 1998) as body composition (BC) has also been demonstrated important in this regard (Bornstein *et al.*, 1984; Soller *et al.*, 1984, Robbins *et al.*, 1986; Robinson and Robinson, 1991). Additionally, Miller and Blyth (1953) found that fasting HP estimates are well correlated with lean or fat-free body mass ($r = 0.92$). This suggests that HP data may be used to estimate lean mass.

An inverse relationship between bird HP and ambient temperature (AT) occurs when AT falls below or exceeds critical limits bordering the thermoneutral (TN) zone (Brody, 1945; Romijn and Lokhorst, 1966; Meltzer *et al.*, 1982; Stanier *et al.*, 1984; Blaxter, 1989). This effect, however, can be potentially offset by bird acclimatization as Li *et al.* (1992) and Wiernusz and Teeter (1993) both observed a HP decline when AT increased, regardless of food intake.

The curvilinear relationship between the fasting HP of a postabsorptive bird at rest in a TN environment, and its live weight is linearized when HP is related to "metabolic body size" (MBS). An appropriate exponent for converting live mass to MBS is attained via the slope of log HP regressed on log BWT (Bender, 1993). Previous studies have proposed the exponent for linearizing BWT and HP at values from 0.75 (Brody and Proctor, 1932; Kleiber, 1947) to 0.67 for poultry (Brody, 1945). Other authors suggest that a more accurate exponent value is obtained when MBS is calculated on a species basis or on a species basis adjusted for age and sex (Thonney *et al.*, 1976; Johnson and Farrell, 1985). This has led to a range of exponent values being applied to poultry.

Using the energy model described by Hurwitz *et al.* (1978), Pinchasov and Galili (1990) calculated maintenance (6.57 kJ/g $BWT^{0.67}$/day) and growth (2.97 kJ/g gain/day) energy needs for 3 to 20 week old broiler pullets. These values are similar to the of 6.74 kJ/g $BWT^{0.67}$/day reported for Leghorn females (Hurwitz *et al.*, 1978), and the 6.07 kJ/g $BWT^{0.67}$/day reported for layers (Bornstein *et al.*, 1979). However Pinchasov and Galili's (1990) estimated energy need for growth was considerably less than the 9.16 kJ/g gain reported by Hurwitz *et al.* (1978) for Leghorn females and the 8.37 kJ/g gain reported by Bornstein *et al.* (1979) for layers. Pinchasov and Galili (1990) speculated that the decrease may be due to genetic and management changes implemented between the late 1970's and 1990, with the 1990 values presumably more accurately describing the modern birds' requirements. Separation of maintenance and growth energy-nutrient needs of limit-fed pullets may allow fine-tuning of ration formulation-consumption allowances to better achieve specific age-body composition combinations.

Historically, BC in food animals has been estimated via proximate analysis. However, since proximate analysis is laborious and necessitates animal slaughter, it is limited in its application. Dual-energy x-ray absorptiometry (DEXA) provides a non-invasive method of monitoring bird BC changes over time. Mitchell *et al.* (1997) evaluated the accuracy of two different DEXA programs vs. proximate analysis, reporting good correlations (r^2 0.90 to 0.97) for the methodologies examined for lean mass. Further, the authors determined that body size as well as the scanning program utilized affected the accuracy of DEXA body fat estimation compared to chemical analysis (R^2 ranging from 0.33 in birds under 2000 g up to 0.73 in birds weighing 2000 g or more). The objectives of the study reported herein were to determine relationships

among HP (in fed and fasted states), BWT, and BC by both DEXA and proximate analysis.

MATERIALS AND METHODS

A 10 X 3 factorial experiment, was conducted utilizing 10 broiler breeder female ages (5-50 weeks) housed at 3 ambient temperatures (AT) (16, 22, and 32 ± 1 C) such that AT-age mediated effects on HP might be related to BWT, BC, and physiological state. Ten commercial broiler breeder parent farms simultaneously supplied the ten age groups (one age per farm) of females from the Cobb female line. Twelve birds were obtained for the 5-, 15-, 20-, 25- and 50-week age groups, while nine birds were obtained for the 10-, 30-, 35-, 40- and 45-week age groups. Experimental group numbers varied to reflect facility limitations. Two sets of data collections, one week apart, were required to complete the measurements. Within each age classifications, birds were selected to fall within 100 grams of the target live fed BWT (Cobb-Vantress, Inc., 1998; Wiernusz, personal communication,1999).

Upon arrival at the research facility, birds were held overnight in the dark at 22 C. The following morning birds were individually placed in open-circuit calorimetry chambers (Figure 1), as per a pre-determined randomization plan. Two sizes of chambers were utilized: twenty-four large calorimetry chambers (53.8 cm X 76.8 cm X 74.2 cm) as well as thirty-six small calorimetry chambers, previously described along with general components and methods (Wiernusz and Teeter, 1993; Belay and Teeter, 1993). Calorimetry chambers were equally distributed in three light and thermostatically controlled rooms with 20 chambers/room (12 small, 8 large). Due to the various ages

utilized in the study, no one lighting schedule was appropriate, therefore, lighting was fixed at 10 hours light and 14 hours dark.

Following chamber placement, birds were fed for three days per breeder recommendations as to amount and composition (Table 1). Feeding levels of 47.5, 48.0, 64.0, 93.0, 118.0, 152.0, 145.0, 141.0, 140.0 and 140.0 g/d were utilized for the bird ages 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 weeks, respectively. Upon completion of the 3-day feeding period, feed was removed for the remainder of the study. Consequently, the study comprised 3 periods; farm selection and transport to research facility, feeding period, and fasting period. Birds were weighed 4 times so that bird weights and weight changes between weighings could be monitored. Live weight was determined on the farm at selection, prior to placement in the calorimetry chambers, when feed was removed at the beginning of the fasting period, and upon completion of the fasting period. All birds had continuous access to water during the study.

Concentrations of O_2 and CO_2 entering and exiting the calorimetry chambers were recorded two times per hour for the smaller chambers and three times per hour for the larger chambers using an Allen-Bradly data acquisition system. Oxygen consumption and carbon dioxide production were estimated as the difference between incoming and outgoing chamber gases, multiplied by the chamber airflow rates. Air flows were: 4491, 6683, 8114, 10116, 12446, 13499, 14159, 14489, 14489 and 14933 ml/min for 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 week old birds respectively. Brouwer's equation (1965) was subsequently used to estimate HP from the calculated O_2 consumption and CO_2 production values. At completion of the experimental period birds were removed from the metabolic chambers, weighed, humanely euthanatized via CO_2 asphyxiation (Smith *et*

al., 1986), double bagged in polyethylene bags, and frozen at -20^0 C until further analysis was performed.

Upon thawing, lean and fat mass as well as skeletal mass and density were estimated using DEXA (Hologic QDR® 1000/W; Figure 2). Previous experience in our laboratory indicated use of the whole body rat mode for scans of birds weighing under 1500 grams and the whole body infant scan mode for birds exceeding 1500 grams for increased accuracy. Following DEXA scans all birds were again double-bagged, frozen and held in a -20^0 C freezer until proximate analysis was performed.

Preparation for proximate analysis (PA) included thawing birds for 3 to 4 hours, placement in covered containers and autoclaving (Amsco® Eagle 3000 Series) for 20 hours at 11 psi (116^0 C) followed by overnight cooling. Each whole bird, including feathers, was then homogenized and dry matter immediately determined. Samples were subsequently assayed for lipid content by ether extract (AOAC International, 1998), bone mass by ashing, and nitrogen and carbon content using the LECO CN-2000 Nitrogen Analyzer.

Statistical analysis of the 10 X 3 factorial treatment arrangement was performed using the General Linear Model (GLM) of the SAS® System (SAS Institute, 1999). Differences among treatments were separated using least square means. The AT, bird age (weight)-HP relationship was examined as a 3-dimensional array to identify the AT closest to TN for each age group. Bird HP, while housed in the presumed TN environment, was subsequently transformed by common logarithm and regressed on log BWT so that the exponent for converting live BWT to MBS might be estimated (Brody and Procter, 1932). Relationships between the two body composition determination

methods were examined by correlation procedure. Linear, non-linear and multiple regression were utilized to develop predictive equations for HP based on body weight and composition and to determine MBS. Paired t-tests were utilized to compare the predicted HP values to actual HP values. Resulting equations relating both composition (lean and fat) and BWT to BMR of non-laying birds were applied to the birds from 30 to 50 wk of age with observations of daily egg production. Resulting HP was compared between hens laying an egg and hens not laying an egg. The resulting difference was compared by paired t-tests to estimate the HP costs associated with the egg formation and oviposition process.

RESULTS AND DISCUSSION

One mortality occurred during the study; the partial data set on this 40-week old bird was excluded from analysis. On farm selection target body weights (Table 2) were met for all ages, save for the 10-week age group whose target fell below the 1100 g desired weight by 100 g. The percentage decrease in BWT from target to placement BWT ranged from 4.5% to 7.8% for the ten age groups.

Body composition was estimated by both the proximate analysis and DEXA methods (Table 3). Proximate analysis provided classical body composition data on a dry matter basis with a whole body water estimate. The total weight of the four components (crude protein, lipid, ash, water) was examined as a percentage of the live fasted BWT and accounted for 98.4% to 99.9% of live mass. The DEXA composition measures included lean, fat and bone mass. Previous work in the authors' laboratory suggests that accuracy might be improved by rescanning when total BWT estimated by DEXA was

outside 5% of the gravimetric mass. In this study all 104 DEXA estimates of BWT were within ± 3.4% of gravimetric mass. The total weight of the three DEXA estimated BC components (lean tissue, lipid, bone mass) accounted for 96.9% to 100.9% of live mass.

Correlation between DEXA and PA composition estimates was examined (Table 4). The highest correlation observed occurred between the DEXA lean tissue and PA protein mass (R=0.9932, $P<0.01$). Lipid mass was quantitatively similar ($P>0.1$) for the DEXA and PA estimates (R =0.9552; $P<0.01$). As such, body fluid may be assigned to the lean mass and enables an estimate of lean tissue dry matter across age. Mean dry matter for lean mass is thereby estimated as 24.7%, calculated as {3 [(PA protein in g)$_{age}$ /(DEXA lean in g)$_{age}$ (100)]}/(10 age groups), and may be used to interconvert PA protein and DEXA lean mass.

Heat production (within an age group) declined ($P<0.05$) as the birds transitioned from fed to fasted states with and without light exposure (Table 5). Since environmental conditions were controlled, the decline reflects a reduced dietary heat increment (Stanier, 1984) and light stimulated activity. Fasted HP per bird was quadratically related to BWT, presumably due to a changing surface area to BWT ratio (kJ/bird/h = 7.07911 + 0.00285 BWT + 9.312 X 10^{-7} BWT^2, R^2=0.99), while HP/kg BWT decreased (kJ/kg BWT/h = 19.94474 – 0.00977 BWT + 1.88 X 10^{-6} BWT^2, R^2=0.93). These HP-BWT measures are in general agreement with published data (Stanier, 1984; Blaxter, 1989; Beker, 1996). Other factors that affect HP include activity (Stanier *et al.*, 1984) and ambient temperature (Blaxter, 1989). The reported HP values collected with the birds in a post-absorptive state at rest in the dark would presumably reflect BMR if housed at TN.

To examine fasted HP (kJ/kg BWT/hr) among weight categories, all birds were assigned to one of nine weight classes. Due to BWT similarity, the 40- and 45-week-old birds (mean BWT of 3.315 vs. 3.332 kg respectively) were examined as one weight group. When HP within a weight-AT group was examined (Table 5), differences attributable to AT were detected. In all but the 1350 g and 2000 g BWT groups (ages 15 and 25 weeks respectively), HP decreased ($P<0.05$) as ambient temperature increased. By definition, HP for birds housed within the zone of thermoneutrality would be minimized. Bender (1993) defined BMR as the HP in a fasted, but not starving, adult animal at rest in a thermoneutral environment. In this study the data surprisingly suggests that the warmest environment ($32^0 \pm 1^0$ C) best represents the thermoneutral AT for all age groups; this AT is considerably higher than the recommended optimum AT range of 18 to 21 ^{0}C (Cobb-Vantress, Inc., 1998). One explanation for this discrepancy may be that the birds became acclimated to the summer conditions on the farms from which they were taken. This explanation is supported by previous work indicating that the thermoneutral zone may change depending on the AT to which birds are acclimated (Balnave, 1974), and other studies in the authors' laboratory have demonstrated that heat stress suppresses BMR.

In order to evaluate these current HP values against historical HP values, three broiler breeder female HP studies, representing a 12-year span, were selected with female breeders that could be matched by BWT to the birds in the current study. In order to minimize the effect of different lighting schedules, the HP values determined in the current study were weighted (e.g. mean by hour current HP_{dark} times hours of dark in historical study) to match each of the selected historical studies. The AT ranges reported

were 20 to 22 °C with an AT range for the current study of 16 to 32 °C. Fasted HP values determined herein (Table 6) were generally lower than those previously reported for all but one case (Balnave *et al.*, 1978; Johnson and Farrell, 1983; Spratt *et al.*, 1990). The trend for lower HP in modern lines may suggest they are more metabolically efficient or may reflect acclimatization to their high AT exposure. Differences between current values and those previously reported were only apparent for fasted HP values. With fed HP values, there was no clear-cut trend even when weighted for lighting schedules.

The exponent to convert live BWT to MBS may be estimated by regressing log HP (kJ/bird/day) on log BWT (kg) with the resulting slope being the exponent. Using the 63 HP values for birds determined to be closest to their thermoneutral environment yielded an exponent of 0.67. This matches Brody's (1945) exponent value of 0.67. As such, the data appears to suggest that heat dissipation of breeder stock has not changed over the past 58 years. Conversely, it may be speculated modern birds either have a surface area to BWT ratio and metabolic rate similar to their 1945 predecessors, or that alterations have been interactively offset.

Regression of fasted HP (kJ/bird/h) on BWT and BC (DEXA) components was performed to create predictive equations (Table 7). When using fasted BWT as the prediction basis, the R^2 observed for the linear equation was somewhat improved by development of a quadratic equation. When using the DEXA components as the prediction basis, a similar improvement of R^2 was observed when multiple regression, using all DEXA components simultaneously, was utilized. Two of the equations, the quadratic based on BWT and the multiple regression based on all three DEXA BC components, were applied herein as a method of ascertaining their reliability. Paired T-

tests examined the two predicted HP values against actual fasted HP for individual birds, where *mu* = (actual HP – predicted HP) = 0. The data were examined with all birds included in one group and with birds separated into age groups. No statistical difference ($P<0.05$) was observed in any of the t-tests. Given these results, it appears that the equations presented herein may be utilized to estimate HP in this strain of broiler breeder females.

Data in the current report enabled estimation of the energy required for egg formation plus oviposition (Table 8). Values predicted for daily BMR by use of either DEXA composition or BWT were in close agreement (and not significantly different) to the observed values when no egg was laid, confirming the validity of this modeling approach. The data indicate that the energy cost of egg formation and oviposition is approximately 45.85 kJ/bird/day.

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Table 1. ***Diet formulations for grower and breeder diets fed to ten female broiler breeder age classes***

	Grower[1]	Breeder[2]
Ingredients	**g/kg**	**g/kg**
Corn	646.12	667.16
Soybean meal (48%)	168.54	220.98
Wheat midds	148.16	0
Phosphate, Ca Di-phosphate	17.188	16.618
Limestone	8.111	65.190
Lysine	7.514	8.252
Methionine	3.300	3.703
Termin-8	3.000	3.000
Fat, animal & vegetable	2.500	18.715
Salt	2.129	1.600
MHA-Alimet	1.006	1.305
Vitamin premix	1.000	1.250
Hygromix	0.750	0.500
Choline	0.728	0.923
Trace minerals	0.600	0.600
Ethoxyquine, dry	0.165	0
Sodium carbonate	0	2.000
Tri-basic copper chloride	0	0.165
ME (kJ/kg)	11.966	12.242
Crude protein (g/kg)	150.0	155.0

[1] Grower fed to birds ages 5, 10, 15 and 20 weeks. [2] Breeder fed to birds ages 25, 30, 35, 40, 45 and 50 weeks.

Table 2. ***Mean (by age) gravimetric body weight (BWT) observed for broiler breeder females ages 5 to 50 weeks***

Age (wk)	5	10	15	20	25	30	35	40	45	50
Target[1] (g)	620	1000	1520	2160	2950	3310	3587	3677	3768	3836
Farm[2] (g)	646	1007	1521[a]	2171[a]	2938[a]	3337	3500[a]	3684	36912	3842
Placement[3] (g)	576	923	1412	2034	2773	3127	3315	3477	3497	3661
% change from Target @ Placement	- 7.0	- 7.8	- 7.1	- 5.7	- 6.0	- 5.6	- 7.7	- 5.5	- 7.2	- 4.5
Begin fast[4] (g)	625	984	1481	2159	3009	3230	3430	3563	3624	3748
End fast[5] (g)	550	884	1348	1993	2800	2972	3171	3315	3332	3491

[1] Target = BWT ± 100 g used to select birds; [2] Farm = Mean BWT at time of selection; [3]Placement = Mean BWT at beginning of fed period; [4]Begin fast = Mean BWT at beginning of fasting period; [5]End fast = Mean BWT at end of fasting period (hour 44 of fast).
[a] BWT at time of selection not recorded by farm personnel, therefore Farm BWT estimated based on linear regression equation. Farm BWT = 49.044 + (1.0424 * Placement BWT). R2 = 0.9991, $P<0.0001$, N = 50 where N represents those birds with both Farm BWT and Placement BWT recorded.

Table 3. ***Mean (by age) group fasted body weight (BWT) and body composition measurements, determined by proximate analysis (PA) and dual-energy x-ray absorptiometry (DEXA), observed in 10 age groups of broiler breeder females***

Age (wk)	5	10	15	20	25	30	35	40	45	50
Number of birds	**12**	**9**	**12**	**12**	**12**	**9**	**9**	**8**	**9**	**12**
Fasted live BWT (g)[1]	552	882	1347	1998	2798	2969	3173	3315	3329	3494
Proximate analysis										
Protein (g)[2]	113	198	313	440	592	623	648	641	661	691
Lipid (g)[2]	34	14	40	154	348	334	412	556	440	510
Ash (g)[2]	17	33	52	76	104	105	112	99	116	118
Water content (g)[3]	386	636	940	1324	1747	1898	1960	1967	2059	2121
Whole bird (g)[4]	550	881	1345	1994	2791	2960	3132	3263	3276	3440
% of live fasted BWT	99.7	99.9	99.9	99.8	99.7	99.6	98.4	98.4	98.4	98.5
DEXA[4]										
Lean (g)	474	797	1227	1677	2351	2518	2636	2643	2817	2843
Fat (g)	68	65	101	212	312	335	418	556	403	518
Bone (g)	11	18	31	47	63	65	74	64	75	76
DEXA BWT(g)	553	880	1359	1936	2726	2918	3128	3263	3295	3437
% of live fasted BWT	100.2	99.8	100.9	96.9	97.4	98.3	98.6	98.4	99.0	98.4

[1] Hour 44 of fast; [2] Dry matter basis, [3] Water content estimate derived through subtraction; [4] As-is basis.

Table 4. *Correlation of broiler breeder female body composition data determined by proximate analysis[1] (PA) and duel-energy x-ray absorptiometry[2] (DEXA) and gravimetric (GRAV) body weight (BWT)*

	Correlation Coefficient		
Composition Components	**(r)**	***P*-value**	**N**
DEXA Lean to PA Protein	0.9932	<0.0001	104
DEXA Fat to PA Lipid	0.9552	<0.0001	104
DEXA Bone to PA Ash	0.9795	<0.0001	104
GRAV BWT to DEXA BWT	0.9996	<0.0001	104
GRAV BWT to PA BWT	0.9891	<0.0001	104
DEXA BWT to PA BWT	0.9888	<0.0001	104

[1] Dry-matter basis; [2] As-is basis

Table 5. ***Fed and fasted heat production (HP) under light and dark conditions determined in ten ages of broiler breeder females housed in three ambient temperatures (AT)***

	Mean HP[1] (kJ/hr) across AT (104 birds)									
Age (wk)	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**
HP (kJ/bird/hr)										
Fed (light)	21.75[a]	22.37[a]	28.27[a]	33.31[a]	47.18[a]	51.76[a]	53.43[a]	52.25[a]	55.27[a]	53.23[a]
Fed (dark)	15.46[b]	14.83[b]	19.44[b]	25.25[b]	35.58[b]	38.80[b]	38.96[b]	37.81[b]	40.61[b]	39.57[b]
Fasted(light)	11.06[c]	13.77[c]	16.85[c]	19.38[c]	26.64[c]	33.72[c]	29.05[c]	34.19[c]	34.23[c]	32.03[c]
Fasted(dark)	8.96[d]	10.29[d]	12.91[d]	15.75[d]	22.15[d]	25.04[d]	24.28[d]	27.42[d]	27.81[d]	27.40[d]
HP (kJ/kg BWT/hr)										
Fed (light)	39.53[a]	25.41[a]	20.99[a]	16.78[a]	16.77[a]	17.36[a]	16.81[a]	15.73[a]	16.58[a]	15.25a
Fed (dark)	28.09[b]	16.84[b]	14.43[b]	12.71[b]	12.69[b]	13.02[b]	12.29[b]	11.40[b]	12.17[b]	11.38[b]
Fasted(light)	20.11[c]	15.63[c]	12.53[c]	9.75[c]	9.51[c]	11.32[c]	9.18[c]	10.31[c]	10.27[c]	9.20[c]
Fasted(dark)	16.26[d]	11.69[d]	9.59[d]	7.93[d]	7.91[d]	8.41[d]	7.66[d]	8.28[d]	8.34[d]	7.85[d]

[a,b,c,d] Means in the same column and within a HP category not followed by a common superscript differ significantly ($P < 0.05$).

	Fasted (dark) HP (kJ/kg BWT/hr) in three ambient temperatures (AT)								
Age (wk)[2]	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40-45**	**50**
BWT (g)[3]	**550**	**880**	**1350**	**2000**	**2700**	**2970**	**3175**	**3315**	**3500**
AT									
16 ± 1.0 C	16.04[e]	11.87[e]	**10.02[e]**	8.61[e]	7.77[e]	10.10[e]	8.44[e]	9.20[e]	7.94[e]
22 ± 1.0 C	19.69[f]	12.53[e]	**9.40[e]**	**7.49[f]**	7.71[e]	8.12[f]	7.91[e]	**8.05[f]**	8.85[f]
32 ± 1.0 C	**12.94[g]**	10.67[f]	**9.36[e]**	**7.70[f]**	**8.23[e]**	**7.00[g]**	**6.63[f]**	**7.57[f]**	**6.77[g]**
BMR[4]	12.94[h]	10.67[i]	9.59[j]	7.59[kl]	7.91[k]	7.00[lm]	6.63[m]	7.83[k]	6.77[m]

[e,f,g] Means in the same column not followed by a common superscript differ significantly ($P < 0.05$).
[h-m] Means in the BMR row not followed by a common superscript differ significantly ($P < 0.05$).

[1]Fed (light) = Mean HP for 4-hr period beginning 2 hr post-feeding, Fed (dark) = Mean HP for 4-hr period beginning 8 hr post-feeding; Fasted (light) = Mean HP for hr 26 through 30 of fasting period, Fasted (dark) & BMR = Mean HP for hr 36 through 40 of fasting period. All means by age and HP period or BWT class and environmental temperature. [2]Due to overlapping fasted BWT range, 40- and 45-week old birds combined into one class, [3]Mean BWT for indicated age groups, [4]BMR = Basal Metabolic Rate, note: values used to calculate mean BMR for each age group, shown in bold-face type, represent HP in the thermoneutral environment as determined for this group of birds.

Table 6. ***Fasted broiler breeder female heat production (HP) as reported in literature and as determined in this study matched by body weight (BWT)***

As reported in literature				As determined this experiment		
Year published	Age (wk)	BWT (kg)	Fasted HP (kJ/kg/h)	BWT (kg)[1]	Age (wk)	Fasted HP (kJ/kg/h)[2]
1990[3]	37 - 40	2.92[a]	9.12	2.97	30	10.10
1990[3]	37- 40	2.81[b]	8.98	2.80	25	8.84
1983[4]	42	3.72[c]	10.83	3.49	50	8.81
1983[4]	42	3.47[c]	10.67	3.49	50	8.81
1978[5]	45	3.3[d]	11.69	3.32	40	9.63
1978[5]	45	3.2[d]	10.94 to 11.79	3.17	35	8.67
1978[5]	45	3.1[d]	12.74	3.17	35	8.67
1978[5]	42	2.9 to 3.0[d]	12.6 to 13.15	2.97	30	10.35

[1] Determined BWT, mean by age, this experiment that most nearly matches reported BWT
[2] Fasted HP determined in this experiment weighted to match lighting schedules used in publications
[3] Spratt *et al* (1990) Poultry Science 69:1348-1356
[4] Johnson and Farrell (1983) British Poultry Science 24:439-453
[5] Balnave *et al* (1978) British Poultry Science 19:583-590.
Genetic stocks utilized in previously reported studies - [a] Hubbard, [b] Arbor Acre, [c] Hyline, [d] Allied Genetic; this experiment - Cobb

Table 7. ***Predictive heat production (HP) equations determined by linear, non-linear and multiple regression for broiler breeder females ages 5 to 50 weeks based on body weight (BWT) and on body composition components***

			Regression Equations		
To estimate fasted HP	**DF**	**R^2**	**Intercept**	**Linear and multiple regression terms**	**Quadratic regression term**
kJ/bird/h	103	0.8022	4.34034	+ 6.53199 fasted BWT	
kJ/bird/h	103	0.8095	7.05996	+ 2.90043 fasted BWT	+ 0.87759 fasted BWT^2
kJ/bird/h	103	0.6760	9.45879	+ 0.03499 fat mass (DEXA)	
kJ/bird/h	103	0.7931	3.81749	+ 0.00806 lean mass (DEXA)	
kJ/bird/h	103	0.8059	4.45366	+ 0.00635 lean mass (DEXA)	
				+ 0.00938 fat mass (DEXA)	
kJ/bird/h	103	0.8081	4.42228	+ 0.00744 lean mass (DEXA)	
				+ 0.00992 fat mass (DEXA)	
				- 0.04391 bone mass (DEXA)	

Table 8. ***Differences recorded between observed and predicted heat production (HP), estimated by either body composition or body weight, as affected by oviposition for 36 broiler breeder hens, ages 30 to 45 weeks***

		Difference between observed and predicted HP (kJ/bird/day)	
Estimation Method[1]	Egg Produced?	Difference	Egg effect
Body weight (BWT; 44 h fast)	Yes (N=11)	51.21*	
	No (N=25)	4.85	
			46.36*
Body Composition (as estimated by	Yes (N=11)	50.92*	
dual-energy x-ray densitometry (DEXA))	No (N=25)	5.58	
			45.34*

*$P \leq 0.05$

[1]Both equations based on HP determined in non-egg laying broiler breeder females ranging in age from 5 to 50 weeks (N=93)
HP (kJ/bird/d) = (8.4560 + 4.163[-1] g fasted BWT + 6.025[-1] g fasted BWT[2])(24 h); $R^2 = 0.898$
HP (kJ/bird/d) = (6.5541 + 6.565[-1] g lean$_{DEXA}$ + 9.083[-1] g fat$_{DEXA}$)(24 h); $R^2 = 0.897$

Figure 1. ***Small (top row) and large (bottom row) metabolic chambers used for collecting heat production data.***

Figure 2: ***Hologic QDR 1000 x-ray densitometer used to estimate whole bird body composition (lean, fat and bone mass).***

CHAPTER IV

RESEARCH NOTE

Metabolism and Nutrition Section

Heat Production Determined by Indirect Calorimetry in Large Broiler Males[1]

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Abbreviation Key: BMR=basal metabolic rate; HP=heat production; MBS=metabolic body size

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ABSTRACT An experiment was conducted to estimate fasted (36 l (HP) during both dark and lighted periods for broiler males of variot Dark phase fasted HP, presumed to represent basal metabolic rate (E curvilinearly with BW. This relationship was linearized via log tran indicated that the appropriate exponent for converting live BW to m 0.66. Lack of change in the determined exponent since 1945 sugges in feed conversion ratio for modern breeds is for reasons other than surface area to mass ratio. A two-hour lighted period, immediately determination, increased HP ($P<0.05$) in all BW groups. Resultant provide a means of estimating HP based on bird BW.

(Key words: broiler breeder males, metabolism, heat production, ce body size)

INTRODUCTION

Although heat production (HP) of male broilers is well-doc Swain, 1977; Koh and MacLeod, 1999), there is a dearth of HP inf literature related to larger male broilers or broiler breeders. Since l metabolism, such information provides insight into energy need an (Wiernusz and Teeter, 1993), body composition (MacLeod, 1997), environmental consequences (Jones, 1994). These areas are impor industry, at both the production and scientific levels.

Basal metabolic rate (BMR), defined as the HP of a fasted a thermoneutral environment (Bender, 1993), has been used as an maintenance energy needs (Ruckebusch et al., 1991). However, s

account for energy cost of nutrient metabolism and activity associated with homeostasis. These values typically increase curvilinearly with BW and linearly with metabolic body size (MBS). Heat production may be determined by either direct or indirect calorimetry (Berdanier, 1995). Brouwer (1965) developed an equation utilizing oxygen consumption, carbon dioxide production, and excretory nitrogen to estimate HP, while Romijn and Lokhorst (1966) suggested that the nitrogen portion could be disregarded as it accounted for less than 0.6% of total HP. MacDonald (1993) validated this approach using comparative slaughter combined with indirect calorimetry.

The objectives of the study reported herein were to estimate HP of large broiler males during BMR and a light induced activity period. Further, objectives of this study were to develop predictive equations relating BW with HP and to estimate the exponent for converting live mass to metabolic body size as a test of the applicability of extrapolating broiler data to the large broiler breeder males.

MATERIALS AND METHODS

Birds used in this study were obtained in a manner that permitted examination of the HP, BW, BMR relationships for a large range of BW. At study initiation there were four BW classes of birds with mean BW of 0.925, 2.28, 4.53, and 5.06 kg, respectively, and there were 9 birds per each BW class (36 birds total). All birds were previously fed a ration containing 20.5% crude protein and 3,190 kcal ME/kg of diet.

Body weight was recorded at the beginning of a 42-h fasting period. At hour 34 of the fast, six birds from each BW group (selected at random) were placed in individual open-circuit calorimetric chambers (53.8 cm X 76.8 cm X 74.2 cm) maintained at 22 °C as previously described (Wiernusz and Teeter, 1993; Belay and Teeter, 1993). The

calorimetric chambers were equally distributed in three light- and thermostatically-controlled rooms. Oxygen consumption and carbon dioxide production were estimated as the difference between incoming and outgoing chamber gases multiplied by the chamber air flow rate. Brouwer's equation (1965) was subsequently used to estimate HP from the determined O_2 consumption and CO_2 production values. Gas data collected during hours 36 to 40 of fasting was in darkness so HP as BMR could be estimated. This was followed by a 2 h lighted period to enable HP measures with greater activity level. Upon completion of HP data collection, birds were removed from the calorimetric chambers, weighed and returned to their group pens. All birds were necropsied at a later time to confirm sex.

Calorimetric data were analyzed using the General Linear Model (GLM) of SAS (1999) to determine light and activity effects on fasted HP. Heat production per hour was regressed on a per bird and a per unit BW basis. Both linear and nonlinear regression was used to determine whether a linear or a quadratic equation provided the better estimate of HP. Finally, log HP was regressed on log BW to determine the proper exponent for converting live BW to MBS as described by Brody (1945).

RESULTS AND DISCUSSION

Upon completion of the BMR determination, necropsy examinations revealed that one 5 kg bird, and one 2.3 kg bird were females; these were excluded from subsequent analysis. An equipment malfunction resulted in the loss of data from one of the three replicate rooms, yielding only 14 birds available for the final analysis. Of these there were 2 from the 0.925, 4 from the 2.28, 3 from the 4.53, and 5 from the 5.06 kg groups, respectively.

Heat production (kcal/h) was examined on a per bird and on a per kg BW basis (Table 1). Regression analysis was used to relate fasted HP (kcal/bird/h) in the dark (presumed to represent BMR) with BW. The quadratic equation resulting from the non-linear regression represents a better fit (R^2 = 0.82) to the data than did the linear equation (R^2 = 0.81). The current observation that HP (kcal/bird/h) increases quadratically with BW agrees with Brody (1945). Furthermore, the decrease in HP (kcal/kg/h) supports previous observations that HP per unit BW is lower for larger animals due to a lower surface area to body mass ratio (Stanier et al., 1984; Blaxter, 1989).

Heat production increased ($P < 0.05$) for birds as they moved from the BMR (fasted HP, dark, and quiet) period to the activity (fasted HP and lights on) period (Table 1). Though each BW class, save the 925 g group, displayed increased activity HP ($P < 0.05$), data for this group deviated less than 5% from unpublished data from the authors' laboratory. It is, therefore, considered reasonable for regression analysis. This increased HP with activity, presumably results from a higher metabolic rate due to increased activity (Balnave, 1974; Blaxter, 1989). In the current study, this increase in HP (kcal/kg BW/h) was 59.2, 50.5, 57.0, and 52.9% for birds weighing 0.925, 2.28, 4.53, and 5.06 kg respectively. Presumably, lighting programs with increased scotoperiod utilize this energy saving to improve feed conversion.

Heat production in kcal/bird/h and kcal/kg/h for both the BMR and the activity periods was regressed on fasted BW using linear and nonlinear regression to develop predictive equations for HP (Table 1). Brody linearized HP with BW by using a logarithmic transformation termed MBS. The exponent to convert live BW to MBS was

estimated by regressing log mean BMR (kcal/bird/d) on log mean BW (kg) and using the slope as the exponent. Using the BMR values for this group of birds:

Log HP (at BMR; in kcal)/bird/d = 4.0128 + 0.6591 log BW (in kg)

$R^2 = 0.89$, $P < 0.0001$

Therefore, the exponent to convert live weight into metabolic body size for this population of male broiler breeders is 0.66, which is a close match to Brody's 1945 value of 0.67.

Regressing log HP for fasted birds housed in the light with increased activity, yields a similar exponent:

Log HP (in light; in kcal)/bird/d = 4.2337 + 0.6573 log BW (in kg)

$R^2 = 0.73$, $P < 0.0001$

The fact that the exponent for converting BW to MBS has not changed since 1945, suggests that improvements in feed conversion are not related to heat dissipation per unit BW. Rather, the feed conversion improvements seen may be due to increased metabolic efficiency of the growth component, enabling fewer days for maintenance, and/or due to better husbandry practices (e.g., lighting schedules, environmental temperature control). Based upon data reported herein, it would appear that factors other than changes in BMR are responsible for alterations in metabolic efficiency of modern birds. In a related study where BMR was estimated for good and poor feed converters (Skinner-Noble and Teeter, 2003), no difference was noted in BMR of good and poor converting broilers, adding additional credence to use of 0.67 as the exponent for converting BW to MBS.

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TABLE 1. Fasted heat production (HP) recorded in broiler breeder males during a basal metabolic rate (BMR) period[1] and during a lighted activity period[2] with regressions equations for estimating HP based on body weight (BW)

Mean BW (kg)	0.925	2.28	4.53	5.06		
N	2	4	3	5		
Mean HP (kcal/bird/h)					Equations for estimating HP ($P < 0.0001$)	R^2
BMR	2.1831[a]	3.9911[a]	6.5048[a]	6.6650[a]	HP = 0.6701 + 1.6907 BW kg – 0.0962 BW kg^2	0.82
Activity	3.4052[a]	6.0036[b]	10.2155[b]	10.2065[b]	HP = 1.448 + 2.1937 BW kg – 0.0804 BW kg^2	0.95
Mean HP (kcal/kg/h)						
BMR	2.3817[a]	1.7550[a]	1.4340[a]	1.3206[a]	HP = 2.8572 – 0.6076 BW kg + 0.0609 BW kg^2	0.81
Activity	3.7918[a]	2.6415[b]	2.2514[b]	2.0189[b]	HP = 4.8156 – 1.256 BW Kg + 0.1417 BW kg^2	0.91

[1] BMR – dark, quiet environment; [2] Activity – lighted with sound simulation. Both at 22° C.

[a,b] Adjacent means within a column with no common superscript differ ($P<0.05$)

CHAPTER V

COMPOSITION DETERMINED BY INVASIVE AND NONINVASIVE METHODS

Education and Production

Estimating Body Composition Utilizing Proximate Analysis, Dual-energy X-ray Absorptiometry, Carbon-Nitrogen Tissue Content and Morphometric Methodologies, and Determining Whole Bird Energy Utilizing Bomb Calorimetry in Broiler Breeder Females [1]

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Abbreviation Key: BC=body composition; CNTC= carbon-nitrogen tissue content; DEXA=Dual-energy x-ray absorptiometry.

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ABSTRACT

The objective of this study was to interrelate several methodologies of determining body composition (BC) in 104 broiler breeder females ages 5 to 50 weeks. Morphological measurements (girth, shank length, and skin-fat pad thickness), dissection of abdominal fat, and technological and laboratory methodologies (dual-energy x-ray absorptiometry (DEXA), proximate analysis, and carbon-nitrogen tissue content) were performed. The morphological BC measurements were related to DEXA estimated lean and fat mass as well as to dissected abdominal fat weight. Girth had the best correlation to DEXA estimated lean mass ($r = 0.94$), while skin-fat pad thickness correlated well with both dissected abdominal fat weight ($r = 0.81$) and DEXA estimated fat mass ($r = 0.86$). Equations for estimating lean mass based on girth measurements and for estimating fat mass based on skin-fat pad thickness were developed (R^2=0.89 and 0.74 respectively).

Dual-energy x-ray absorptiometry and classical proximate analysis (PA) methods were used to determine lean, fat and bone mineral mass for each bird. The carbon-nitrogen tissue content (CNTC) and whole bird energy were determined for the same birds. Statistical analysis determined that good relationships ($r > 0.95$) existed among three methodologies, PA, DEXA, and CNTC, for each type of body component. Equations for interrelating these three methodologies ($R^2 \geq 0.90$) and for estimating whole bird energy based on CNTC (R^2 0.88) were developed.

Key words: body composition, carbon-nitrogen tissue content, dual-energy x-ray absorptiometry, female broiler breeders, proximate analysis

INTRODUCTION

Accurately determining body composition (BC) in food animals has been a prime objective of researchers with numerous methods utilized historically (Hedrick, 1983; Robinson et al, 1996). Proximate analysis (PA) is the conventional method for assaying tissue samples for lean, lipid and mineral, as ash, levels and considered to be the "gold standard" (Blaxter, 1989). Carbon-nitrogen balance (CNB) has been utilized as a method for assaying changes in tissue levels of lean and lipid (Blaxter and Rook, 1967; Farrell, 1974). Assuming CNB effectively quantifies lean and lipid changes over time, then one should be able to use carbon-nitrogen tissue content (CNTC) to estimate tissue composition. Although both CNTC and PA require animal sacrifice, determining the CNTC would be a less laborious method than PA given the current availability of automated carbon-nitrogen analyzers.

In vivo assessment of BC in food animals is an important concern to both breeders and growers since lean to fat ratios and mass directly relate to product value. Additionally, the ability to track changes in BC over time in the same animal would be useful to researchers. However, determining the proportion of lean and fat in live animals is difficult at best. Robinson et al (1996) measured various body components (shank length, head width, keel length, and girth) as a method of assessing BC in broiler breeder hens that had been photostimulated at five different ages. Other than for girth, they observed no differences ($P< 0.05$) among groups at trial end (bird age 60 weeks). Pym and Thompson (1980) developed a caliper methodology wherein abdominal fat pad thickness was used to estimate abdominal fat weight. They calculated the correlation between estimated abdominal fat weight and actual abdominal fat weight to be 0.76 for

male broilers and 0.75 for females. Other studies have shown a correlation between abdominal fat weight and total body fat with correlation coefficients ranging from 0.60 to 0.81 (Chambers and Fortin, 1984; Becker et al, 1981). Therefore being able to accurately estimate abdominal fat weight in vivo would be a good indicator of total body fat.

Dual-energy x-ray absorptiometry (DEXA) is a non-invasive approach for estimating lean, lipid and bone mineral levels in tissue (Mitchell et al, 1996; Mitchell et al, 1997). Although requiring the animal being studied to be anesthetized, DEXA allows the tracking of BC changes over time in the same animal. This supplies more accurate data to the researcher than using comparative slaughter data, which, historically has been an accepted method for determining BC changes over time for a group of animals.

MATERIAL AND METHODS

Preliminary experiment

A preliminary study was conducted to determine which autoclave cycle provided a sample that most closely matched the nitrogen value of raw tissue. Breast tissue from each of three birds was minced and homogenized. Subsamples from each prepared sample were autoclaved[1] for 8, 14, 20 or 24 hours at 11 psi (240^0 F). Nitrogen values for both autoclaved samples and raw samples were determined with an automated analyzer[2]. Statistical analysis was performed to determine which autoclave cycle yielded a nitrogen value that most closely matched that determined for the raw samples.

Experiment

Broiler breeder females varying in age, 5 to 50 weeks, were selected at parent farms to represent a growth-curve range. All birds were raised according to breeder

recommendations (Cobb-Vantress, Inc., 1998) for feeding and lighting. Birds were selected to fall within +/- 100 grams of the target live fed BW for a given age (Cobb-Vantress, Inc., 1998; Wiernusz, personal communication,1999). After transport to the experimental location, birds were housed individually and refed, according to breeder guidelines, for 72 hours before beginning a fasting period. Water was available ad lib throughout the trial.

Body weight (BW) was recorded at the beginning and the end of a 44-hour fasting period. Upon completion of the fast, in vivo morphological measurements were performed. Shank length (cm), right leg only, and girth measurements (cm) were made as described elsewhere (Robinson et al, 1996). Abdominal skin-fat pad pinch measurements (mm) were performed with the bird held facing backward in an upright position under the left arm of the researcher. A pinch of abdominal skin, including any underlying fat pad, location as previously described by Mirosh et al (1980), was gently grasped between the thumb and first finger of the left hand followed by placement of the caliper[3] arms immediately caudal to the left hand fingers. Shank and girth measurements were taken twice while three skin-fat pad pinch measurements were made; mean values were used for analysis. Upon completion of morphometric measures, birds were humanely euthanatized by CO^2 asphyxiation (Smith et al, 1986), and were individually double-bagged in polyethylene bags and frozen at -20^0 C. The composition of these birds was subsequently estimated by three methods, DEXA, PA and CNTC. First, birds were thawed, weighed and DEXA[4] scans performed to provide an estimate of lean tissue, fat and mineral mass. Following the scans, birds were replaced in polyethylene bags and frozen at -20^0 C until PA was scheduled. After partial thawing (3 to 4 hours), each bird

was placed in an autoclave-safe container, covered and autoclaved for 20 hours at 11 psi (240^0 F) followed by overnight cooling. Weights were recorded before and after autoclaving. Each whole bird was homogenized, including feathers; birds over 800 grams were homogenized in a Waring commercial grade blender with a one gallon container while birds less than 800 grams were homogenized in a household grade Black & Decker food processor. The appropriate samples were taken for conventional PA (AOAC, 1998); dry matter was determined for each sample. Lipid content was determined by ether extraction (AOAC, 1998). Mineral content was calculated based on change in sample weight after ashing for 6 hours at 500^0 F. Total body nitrogen and carbon were determined using an automated carbon-nitrogen analyzer[5]. Whole bird energy was determined with an adiabatic bomb calorimeter[6]. Duplicate samples were run for all analyses and mean values used for subsequent statistical analysis.

Statistical analysis (SAS, Inc., 1999) included correlation of BC components among the methodologies. Linear regression was applied to those sets of BC measurements where it was reasonable to use one variable as the basis for estimation. For those methodologies generating more than one BC component, multiple regression was applied to determine the best equations for estimating a BC component generated by another methodology.

[1] Amsco© Eagle 3000 Series Autoclave, Steris Corp., Mentor, OH.
[2] Kjeltec 2400 Analyzer Unit, Foss Tecator AB, Höganäs, Sweden.
[3] Harpeden Skinfold Calipers, Model 0120, General Tools Manufacturing Co., Inc., New York, NY.
[4] Hologic QDR® 1000/W DEXA, Hologic, Inc., Waltham, MA
[5] LECO CN-2000, LECO©Corp., St. Joseph, MI.
[6] Isoperibol Calorimeter Model 1261, Parr Instrument Co., Moline, IL.

RESULTS AND DISCUSSION

Preliminary experiment

Comparison of nitrogen values for the preliminary samples revealed that the 20-hour autoclave cycle yielded a sample with nitrogen values closest to that of the comparable raw sample (Table 1). Additionally, the percent nitrogen in both of the 20-hour samples is comparable to values previously reported for the nitrogen content on a dry matter basis in chicken breast of 14.66% in 6-week-old and 15.06% in 1-year-old Leghorns (Zarkadas et al, 1987).

Experiment

Due to non-availability, 32-week old birds were substituted for 30-week old birds; however, the selection was based on target BW for 30-week old birds. Target body weights were met for all age groups except for the 10-week old birds; the target BW for this group had to be lowered by 100 g in order to obtain sufficient birds. One mortality occurred during the study; the partial data set on this 40-week old bird was excluded from analysis.

Morphological Measurements. Of the three morphological measurements, girth, shank length, and abdominal skin-fat pad pinch thickness (Table 2), girth had the best correlation with DEXA lean tissue mass ($r=0.95$). Girth circumferences in this study were similar to those observed by Latshaw and Bishop (2001) in broilers when birds of similar BW were compared. Using data generated by this experiment, a high correlation ($r = 0.955$) between fat mass determined by DEXA and lipid content determined by PA was determined. Skin-fat pad pinch thickness had good correlations with dissected abdominal fat weight and with DEXA fat weight, $r=0.81$ and $r=0.86$ respectively. This

correlation between skin-fat pad thickness and dissected abdominal fat weight is substantially higher than the range (r=0.29 to 0.54) reported by Mirosh and Becker (1982) using the Pym and Thompson (1980) caliper method. Having a technique, such as the skin-fat pad pinch measurement, to estimate the amount of whole body fat mass *in vivo* would be useful to both poultry growers and researchers. The technique used in this study is less invasive than the Pym and Thompson (1980) technique, and, if performed by the same operator, appears to supply a moderately accurate estimate of abdominal fat weight. The best correlation observed for shank length was with lean tissue mass estimated by DEXA (r=0.72). Although this correlation coefficient would not encourage using shank length as a stand-alone component of a predictive regression equation, it could serve as a component of a multiple regression model (Reid et al, 1984).

Two equations, based on girth and skin-fat pad pinch measurements, were developed to estimate whole body lean mass and fat mass respectively.

$$\text{Lean mass}_{DEXA}\,(g) = -1919.562 + 111.529\ \text{girth}$$
$$(p<0.0001;\ r^2=0.8934)$$

$$\text{Fat mass}_{DEXA}\,(g) = 37.093 + 34.916\ \text{skin-fat pad}$$
$$(p<0.0001;\ r^2=0.7372)$$

Using these two low-technology methods, in conjunction with BW, would allow quick and easy estimates to be made in the field for tracking changes over time in the lean and fat mass. This information could be useful to both growers and researchers for making feeding decisions or tracking actual gains against expected gains.

Technological and laboratory methodologies. Body composition was estimated by three methods, DEXA, PA and CNTC analysis with bomb calorimetry providing an estimate of whole bird energy (Table 3). Classical BC data (crude protein, lipid and ash)

on a dry-matter basis was provided through PA. The water content of the PA samples was determined through subtraction of the three BC components from the post-autoclave gravimetric BW. Each of the BC components was examined as a percentage of the post-autoclave BW. The resultant percentages are very similar to those determined by Latshaw and Bishop (2001) for broilers fed ad lib when the data for similar size birds were compared; the BW for ages 15 and 20 weeks in this experiment were closest to the published BW range of 1154 to 2456 g. As would be expected, since the birds in this experiment were limit-fed, the mean lipid values were lower (3.0% and 7.7% compared to 13.5%) with higher values observed for protein (23.3% and 22.1% compared to 19.9%) and ash (3.9% and 3.8% compared to 2.7%). Both the protein and ash percentages for a 2500 g hen, 22.1% and 4.2% respectively, as presented by Blaxter (1989), are higher than the Latshaw and Bishop values and more in line with the values seen in this experiment, 21.2% for protein and 3.7% for ash, for similar size birds. Additionally, the post-autoclave weight was examined as a percentage of live fasted BW and accounted for 98.4% to 99.9% of live mass.

An estimate of three BC components (lean, fat and bone mass) on an as-is basis was provided through DEXA (Table 3). Previous work in the authors' laboratory suggests that accuracy might be improved by rescanning when total BW as estimated by DEXA was outside 5% of the gravimetric mass. In this experiment, the DEXA estimates of BW for all 104 birds were within $\pm$ 3.4% of the gravimetric mass. Additionally, the DEXA estimates of BW were examined as a percentage of live fasted BW and accounted for 96.9% to 100.3% of live mass.

The CNTC, as percentages, was determined for each whole bird homogenized sample using an automated CN analyzer (Table 3). These percentages, when compared with similar size birds, are comparable to those determined by McDonald and Teeter (1993) for broilers (BW range of 985 g to 1307g) of 59.4% carbon and 10.51% nitrogen. The lower carbon values seen in this experiment are most likely due to the fact that these birds were limit-fed resulting in a higher protein to fat ratio. Therefore, since protein has a lower percentage of carbon than fat, 52.0% and 76.7% respectively (Farrell, 1974), it would be expected that limit-fed birds would have a lower carbon percentage. Nitrogen (N) and carbon (C) mass (dry-matter basis) were calculated as follows:

N or C (g) = [(Post-autoclave BW)(fraction dry-matter)](fraction N or C)

An estimate of protein (dry-matter basis) was made by multiplying the calculated nitrogen mass by 6.25, the generally accepted value for this calculation (Blaxter, 1989). A comparison of calculated protein values from PA and CNTC methodologies revealed only a small percent difference between the two methods from a low value of 0.02% (30-week old birds) to a high value of 3.0% for the 5-week old birds.

Whole bird energy was determined with an adiabatic bomb calorimeter (Table 3). An examination of the literature found no comparable data on whole bird energy; only data on the energy content of carcasses were available. A comparison of the carcass energy value determined by Wiernusz et al (1999) of 6150 cal/g for broilers with a BW range of 343 g to 2387 g was made with similar size birds in this experiment. It was found that the energy content based on the whole bird (i.e. to include viscera, head, feet and feathers) was lower with a range 4717 to 5973 cal/g for birds through age 25 weeks.

This finding is not unreasonable since it is to be expected that the energy of carcasses would be more concentrated than the energy content of a whole bird.

Data suggests that the three methodologies (PA, DEXA, CNTC) are measuring comparable mass. Correlation analysis of the components supports this observation with correlation coefficients ranging from 0.8089 between bone $mass_{DEXA}$ and $lipid_{PA}$ to 1.00 between $protein_{PA}$ and $nitrogen_{CNTC}$ (Table 4). In order that the three procedures might be interrelated, multiple regression was performed on the variables such that the three techniques could be interrelated (Table 5). Additionally, an estimate of energy was made based on whole bird CNTC. An examination of the ANOVA values for these equations (in all cases $P<0.0001$ and $R^2 \geq 0.8778$) suggests that these equations may be reliably used to estimate the indicated BC components.

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TABLE 1. Nitrogen content determined for raw and autoclaved (240^0 F, 11 psi) minced broiler breeder breast tissue samples using four autoclave cycle lengths

Autoclave cycle length (h)	8	14	20	24
N% Dry matter basis				
Raw	14.71	14.62	15.36	14.76
Autoclaved	15.02	15.15	15.37	14.55
P-value[1]	0.0038	0.0004	0.9658	0.2099
n	18	18	17[2]	18

[1] Statistical analysis performed using GLM procedure of SAS® System, SAS Institute, 1999; [2] One sample discarded due to procedural error during dry matter sample preparation

TABLE 2. Fed and fasted body weights and body composition measurements determined by morphometric measures and dissection observed in broiler breeder females

		Body weight (g)				Morphometric measurements						Dissection	
		Fed		Fasted		Girth (cm)		Shank (cm)		Skin-fat pad (mm)		Abdominal fat (g)	
Age (wk)	N	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean*	Range
5	12	624	584, 667	552	510, 592	21.0	19.5, 22.7	6 1	5.6, 6.5	1.9	1.3, 3 1	2 6[1]	0 7, 6 7
10	9	964	917, 1019	882	797, 917	24 7	23 6, 25 8	7.5	7.2, 8.0	1 8	1.3, 3 0	0[2]	0, 0
15	12	1481	1431, 1512	1347	1250, 1388	30.5	28.5, 33.5	8.4	4.3, 8.8	3.6	2.1, 4.2	1.8[3]	1 0, 2.4
20	12	2159	2000, 2391	1998	1864, 2165	34.2	31.9, 36.6	8.4	7.7, 8.9	5.2	3.6, 6.6	16.7	4 4, 33.2
25	12	3009	2869, 3135	2798	2622.2941	39.2	36.0, 53.6	8 4	8.0, 8.7	7 5	4.0, 9.9	65.4	22 1, 105.8
30	9	3230	3065, 3397	2969	2874, 3058	39 4	37 3, 40.3	8 4	8.2, 8.8	9.0	6.4, 18.1	65.7	27 5, 115.9
35	9	3430	3221, 3569	3173	3033, 3367	39.8	38.5, 41.2	8.3	7.9, 8.8	10.9	5.9, 17.8	86.1	25.2, 125.0
40	8	3563	3407, 3636	3315	3223, 3436	39.7	37.8, 42.2	8.2	7.2, 8.7	13.8	10.0, 19.7	131.3	96 3, 214.8
45	9	3624	3374, 3810	3329	3170, 3451	4 0.9	39.2, 47.1	8.3	7.8, 8.7	10.9	8.7, 14.7	94.3	51 6, 148.1
50	12	3748	3556, 3924	3494	3311, 3689	40 8	38.6, 42.8	8.6	8.3, 9.1	10.5	5.2, 15.2	117.1	42.9, 188.2

* Mean calculated on measurable fat in [1] 6 of 12 birds, [2] 0 of 9 birds, [3] 3 of 12 birds.

Table 3. Mean (by age) body composition measurements determined by proximate analysis (PA), dual-energy x-ray absorptiometry (DEXA), and carbon-nitrogen tissue content (CNTC) analysis with whole bird energy determined by bomb calorimetry, observed in broiler breeder females

Age (wk)	5	10	15	20	25	30	35	40	45	50
Number of birds	12	9	12	12	12	9	9	8	9	12
Proximate Analysis										
Nitrogen (g.dry matter)	18.1	31.7	50.1	70.4	94.6	99.6	103.7	102.5	105.7	110.6
% of gravimetric BW	3.3	3.6	3.7	3.5	3.4	3.4	3.3	3.1	3.2	3.2
Protein[1] (g. dry matter)	113.4	197.9	313.3	440.1	591.4	622.6	648.4	640.9	660.7	691.5
% of gravimetric BW	20.5	22.5	23.3	22.1	21.2	21.0	20.7	19.6	20.2	20.1
Lipid (g. dry matter)	34.0	14.2	40.0	154.0	347.4	334.1	412.2	556.3	439.8	510.0
% of gravimetric BW	6.2	1.6	3.0	7.7	12.5	11.3	13.2	17.1	13.4	14.8
Ash (g. dry matter)	16.9	32.5	51.4	75.7	104.3	104.7	112.1	98.9	115.8	117.5
% of gravimetric BW	3.1	3.7	3.9	3.8	3.7	3.6	3.6	3.0	3.5	3.4
Water content[2] (g)	386	636	940	1324	1747	1898	1960	1967	2059	2121
% of gravimetric BW	70.2	72.2	69.9	66.4	62.6	64.1	62.6	60.3	62.9	61.7
Gravimetric BW[3] (g. as- is)	550	881	1345	1994	2791	2960	3132	3263	3276	3440
% of live fasted BW	99.7	99.9	99.9	99.8	99.7	99.6	98.4	98.4	98.4	98.5
DEXA										
Lean (g. as-is)	473	796	1227	1677	2351	2518	2636	2643	2817	2843
% of DEXA BW	85.7	90.6	90.3	86.6	86.2	86.3	84.3	81.0	85.5	82.7
Fat (g. as-is)	68	65	101	212	312	335	418	556	403	518
% of DEXA BW	12.3	7.4	7.4	11.0	11.4	11.5	13.4	17.0	12.2	15.1
Bone (g. as-is)	11	18	31	47	63	65	74	64	75	76
% of DEXA BW	2.0	2.0	2.3	2.4	2.3	2.2	2.4	2.0	2.3	2.2
DEXA BW (g. as-is)	553	880	1359	1936	2726	2918	3128	3263	3295	3437
% of live fasted BW	100.2	99.8	100.9	96.9	97.4	98.3	98.6	98.4	99.0	98.4
CNTC										
Percent nitrogen	10.9	12.9	12.3	10.5	9.1	9.3	8.6	7.6	8.3	8.1
Nitrogen (g. dry matter)	17.6	31.2	50.5	71.1	94.2	99.6	103.5	101.3	104.6	109.3
Protein[1] (g. dry matter)	110.0	195.2	315.6	444.6	588.7	622.7	646.8	633.0	654.0	683.4
Percent carbon	51.9	47.1	48.0	51.9	54.9	54.8	56.2	58.7	56.4	57.2
Carbon (g. dry matter)	83.7	114.1	196.6	354.1	575.7	587.8	680.8	781.9	710.1	777.4
Bomb calorimetry										
Whole bird energy (cal/g)	5441	4717	4899	5511	5973	5910	6110	6485	6200	6277

[1] Protein (g) estimated by multiplying grams nitrogen by 6.25. [2] Water content estimate derived through subtraction. [3] Post-autoclaving.

TABLE 4. Correlation of body composition values determined by proximate analysis[1] (PA), dual-energy x-ray absorptiometry[2] (DEXA) and tissue analysis for carbon-nitrogen content[1] in 104 broiler breeder females

	Correlation Coefficient (r)				
	Tissue levels - nitrogen		Tissue levels - carbon	*P*-value	N
DEXA Lean	0.9919		0.9589	<0.0001	104
DEXA Fat	0.8552		0.9506	<0.0001	104
DEXA Bone	0.9488		0.8900	<0.0001	104
PA Protein[3]	1.0000		0.9490	<0.0001	104
PA Lipid	0.8754		0.9797	<0.0001	104
PA Ash	0.9735		0.9044	<0.0001	104
	PA Protein[3]	PA Lipid	PA Ash		
DEXA Lean	0.9932	0.8914	0.9618	<0.0001	104
DEXA Fat	0.8620	0.9552	0.8182	<0.0001	104
DEXA Bone	0.9514	0.8089	0.9795	<0.0001	104

[1] Dry-matter basis, [2] As-is basis; [3] Protein (g) estimated by multiplying grams nitrogen by 6.25

Table 5: Body composition (BC) in broiler breeder females, ages 5 to 50 weeks, estimated by proximate analysis (PA), dual-energy x-ray absorptiometry (DEXA), and carbon-nitrogen tissue content (CNTC)

To estimate:	Basis for BC Estimate - Proximate Analysis (dry-matter)				ANOVA*
BC DEXA (as-is)	Intercept	g Protein	g Lipid	g Ash	R^2
Lean tissue (g)	4.133	+ 4.053	+ 0.315	- 1.116	0.9881
Fat (g)	40.613	- 0.044	+ 0.786	+ 0.677	0.9157
Bone mineral (g)	- 1.401	- 0.009	+ 0.003	+ 0.689	0.9596
To estimate:	Basis for BC Estimate - DEXA (as-is)				ANOVA*
BC PA (dry matter)	Intercept	g Lean tissue	g Fat	g Bone Mineral	R^2
Protein (g)	19.932	+ 0.201	+ 0.028	+ 1.199	0.9891
Lipid (g)	- 92.376	+ 0.151	+ 0.856	- 3.453	0.9514
Ash (g)	1.014	+ 0.018	- 0.018	+ 0.999	0.9756
To estimate:	Basis for BC Estimate – CNTC (dry matter)				ANOVA*
Grams of	Intercept	g Carbon	g Nitrogen		R^2
Protein, PA (dry-matter)	1.992^{-13}	-1.029^{-14}	+ 6.250		1.0
Lean, DEXA (as-is)	28.435	+ 0.568	- 21.588		0.9870
Lipid, PA (dry-matter)	- 6.766	+ 1.142	- 3.396		0.9894
Fat, DEXA (as-is)	40.376	+ 0.954	- 2.627		0.9259
Ash, PA (dry-matter)	- 5.676	- 0.027	+ 1.294		0.9514
Bone, DEXA (as-is)	- 4.756	- 0.010	+ 0.789		0.9012
To estimate:	Basis for Energy Estimate – CNTC (dry matter)				ANOVA*
Calories/g of	Intercept	g Carbon	g Nitrogen		R^2
Energy	5126.232	+ 3.802	- 15.506		0.8778

* In all cases, DF = 103 and $P<0.0001$

CHAPTER VI

RESEARCH NOTE

Education and Production

Relationship between Body Composition Components Estimated by Ultrasound and Dual-energy X-ray Absorptiometry Compared to Selected Dissected Body Components in Broiler Breeder Females and Males

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Abbreviation Key: BC=body composition; DEXA=Dual-energy x-ray absorptiometry; U/S=ultrasound.

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ABSTRACT Two experiments were conducted to examine body composition (BC) of broiler breeder males and females over a range of body weights. Dual-energy x-ray absorptiometry (DEXA) scans provided estimates of lean mass, fat mass, bone mass and bone density, whereas cross-sectional measures of breast muscle (males and females) and fat pad (females only) were provided through ultrasound (U/S). Dissection provided direct measures of breast mass and abdominal fat mass. Correlation analysis revealed strong correlation coefficients ($r \geq 0.88$ in males; $r \geq 0.92$ in females) between U/S, DEXA and breast mass components. A poor correlation ($r = 0.22$, females only) was found between mean fat pad thickness (U/S) and abdominal fat weight. Multiple regression analysis yielded equations ($R^2 \geq 0.92$ in males; $R^2 \geq 0.94$ in females) enabling lean tissue and breast mass estimates using U/S measurements. This study suggests that in vivo U/S measurements may be use to estimate lean tissue mass and breast mass in broiler breeders.

(Key words: broiler breeders, body composition, ultrasound, dual-energy x-ray absorptiometry)

INTRODUCTION

Assessing live body composition (BC) of food animals is an important concern since lean to fat ratios and mass directly relate to product value. Historically various methods have been used to assess animal BC with inconsistent results (Hedrick, 1983) with a wider range of methods being proposed as technological advances have been made (Topel and Kauffman, 1988). Ultimately, the standard for measuring BC is proximate analysis. However, since this method requires animal sacrifice and homogenization is

laborious, non-invasive methods that allow rapid accurate in vivo measurement of compositional mass are needed.

Two noninvasive BC assessment methods include ultrasound (U S) and dual-energy X-ray absorptiometry (DEXA). The beef industry has used U/S in vivo to ascertain ribeye size and backfat thickness in cattle for a number of years (Brethour, 1989; Henderson-Perry et al., 1989; Brethour, 1990;). High correlation (r 0.975) has been reported (Brethour, 1992) for consecutive U/S measurements on the same animal by the same operator. Mitchell et al. (1996, 1997) evaluated DEXA measurements in growing pigs and growing chickens. The authors observed that the pig results were viable for determining BC changes over time, while bird mass appeared to affect accuracy in the chicken study. An assessment of the accuracy of DEXA compared to proximate analysis for estimating BC in broiler breeder females found a high correlation ($r \geq 0.96$) between all body components as estimated by DEXA and body components as estimated by proximate analysis (Dixson and Teeter, 2003, submitted for publication).

The objective of the study reported herein was to estimate the lean tissue mass and breast mass of both male and female broiler breeders over a range of body weights utilizing both noninvasive and invasive methodologies. The noninvasive methods utilized were U/S and DEXA; the invasive methodology was dissection of the breast muscles (males and females) and fat pad (females only). Additionally, the relationships among the methodologies were to be determined with the objective being to develop equations to accurately estimate lean tissue mass and breast mass given information from the U/S methodology.

MATERIALS AND METHODS

Experiment One

An experiment was conducted to evaluate using U/S as an estimator of three BC components (breast mass, total lean tissue mass and abdominal fat mass) in a group of pullet-breeder hens over a range of ages and body weights (BW). One hundred four birds were selected from ten commercial broiler breeder parent farms containing different age groups of females from the Cobb female line. Birds were selected to fall within 100 grams of the target live fed BW (Cobb-Vantress, Inc., 1998; Wiernusz, personal communication, 1999) for the bird age at each farm. All birds were raised according to breeder recommendations (Cobb-Vantress, Inc., 1998) for feeding and lighting. After transport, birds were individually housed, then fed (per breeder recommendations) for 72 hours prior to a fasting period. Water was available ad lib throughout the experiment.

At the completion of a 44-hour fast, all birds were humanely euthanized via CO_2 asphyxiation (Smith et al, 1986), double bagged in polyethylene bags and frozen at -20^0 C until analysis. Upon thawing, birds were scanned using U/S (Figure 1).[1] Scans consisted of three measurements (Figure 2) of both left and right *Superficial pectoralis* and *Supracoracoideus* muscles for a total of six breast measurements per bird. The U/S software allowed an image to be frozen and end-points indicated for each of the three breast measurements. Breast feathers were plucked when required to facilitate full contact between the probe head and skin; a water-based U/S transmission gel was applied to probe head prior to contact with skin. Additionally, three abdominal fat pad measurements were performed per bird (Figure 2). Abdominal feathers were plucked as

[1] Dynamic Imaging Concept MLV Veterinary U/S Scanning System with a 10 MHz Tendon Probe. Products Group International, Inc., Lyons, CO.

needed in order to perform the fat pad measurement. Two fat pad measurements were taken for each location and the mean thickness for each of the three locations used for statistical analysis. All measurements were performed with the bird placed on its back.

Lean, fat and bone mass was estimated using DEXA.[2] For increased accuracy, based on information from previous studies at our facility, the whole body rat mode was utilized for scans of birds weighing under 1,500 grams and the whole body infant scan mode used for scans of birds exceeding 1,500 grams. Scans were repeated if total BW, as estimated by DEXA, deviated more than ± 5.0% from gravimetric BW mass. Both the left and right breast muscles and the abdominal fat were excised and weights recorded.

Body weight was monitored prior to U/S and DEXA measures (i.e., taken after thawing and before U/S scans; taken after thawing and before DEXA scans). These weights were then compared to the live fasted BW.

Experiment Two

Twenty-three broiler breeder males were selected to provide a range of BW in three BW classes. All birds were previously fed a proprietary diet (20.5% crude protein and 3,190 kcal/kg) ad libitum since hatching. Water was available ad libitum throughout the growing period.

The gravimetric BW was taken immediately prior to in vivo U/S breast measurements being performed. The measurements performed were the same as used with the female birds (Figure 2). An assistant was utilized to manually restrain the wings and feet with the bird placed on its back. Fat pad measurements were not made because

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[2] Hologic QDR® 1000/W DEXA. Hologic®, Inc., Waltham, MA

of the poor correlation found in the first experiment between U/S fat pad thickness and actual abdominal fat weight.

To minimize BC changes between methodologies, birds were humanely euthanized immediately following the U/S procedure via CO_2 asphyxiation (Smith et al, 1986), placed in polyethylene bags, and frozen at – 20^0 C. After thawing, DEXA[2] scans were performed to estimate lean, fat and bone mass. The same DEXA scanning procedures used in the first experiment were utilized. Following scanning, breast tissue was excised and the weights recorded for both left and right sides.

Both Experiments

Statistical analysis of data collected was performed using the SAS System (SAS® Institute, 1998). Correlation and regression analysis examined relationships of the two body composition measurement methodologies, U/S and DEXA, relative to each other and dissected breast mass so that predictive equations might be developed.

RESULTS AND DISCUSSION

Experiment One

Body weights prior to U/S and DEXA measures were correlated with live fasted BW and to BW as estimated by DEXA. In all cases, a good correlation ($r > 0.99$, $P < 0.0001$) was observed.

Body composition (lean, fat and bone mass) was estimated using DEXA (Table 1). Previous work suggests that accuracy can improved by using DEXA estimated BW as a test. When total BW (DEXA) was within ± 5% of the gravimetric BW the scan was acceptable. In this study all 104 BW (DEXA) were within ± 3.4 % of gravimetric BW.

Other data have revealed a good correlation (r = 0.99) between lean tissue mass (DEXA) and total body protein determined by proximate analysis (Svendsen et al, 1993; Dixson and Teeter, 2003, submitted for publication). Therefore, the decision was made to accept the lean tissue mass (DEXA) data in this trial as an accurate assessment of whole bird protein.

All six planned U/S breast measurements (Table 1), three per side (Figure 3), were completed on 103 of 104 birds. Correlation was utilized to determine the relationships between the U/S measurements and lean tissue mass (DEXA) and dissected breast mass (Table 2). Using mean values of matched U/S measurements (i.e. left-side and right-side values for the same measurement) equaled or improved the correlation coefficient determined for a single-side measurement. Best-fit correlation variables were used to develop regression equations, based on the U/S measurements, enabling prediction of dissected breast mass and DEXA lean tissue mass (Table 3). In an experiment examining the relationship of breast muscle mass to U/S measurements, Rémignon et al (2000) observed that breast muscle area (determined using U/S images) in conjunction with BW was a good predictor of breast muscle mass (R^2 0.84 in one experiment and R^2=0.78 in a second experiment) in male broilers. However, calculating the breast muscle area had to be performed at a later date using a software program not integral to the U/S programming. In contrast, the determination of the breast muscle measurements in this experiment was integral to the U/S software; this allows the calculation of breast muscle mass, using the regressions equations given herein, to be made immediately if desired.

Due to small bird size (i.e. abdominal area too small for U/S probe head size), U/S measurements of the abdominal fat pad could only be performed on 50 of the 104 birds (BW $\geq$ 2900 grams). Abdominal feathers had to be plucked on most birds in order to perform the fat pad measurement. Care was taken to prevent compression of the fat pad; this compression could be observed on the U/S screen when the U/S probe was applied to the abdomen. Correlation of mean fat pad thickness (U/S) to abdominal fat weight revealed no significant relationship ($r = 0.22480$, $P = 0.1204$).

The data from this experiment indicate that lean tissue mass (DEXA) and breast mass (dissected) may be reliably estimated based on U/S measurements of the *Supracoracoideus* muscle in broiler breeder females. Since U/S is non-invasive, this methodology would be suitable for use in vivo. Additionally, since there are portable U/S units available (e.g. the one utilized herein was approximately 25 kg, including carrying case), this methodology could be used in field situations.

Experiment Two

All six in vivo U/S breast measurements completed on each of the 23 male birds were used to calculate the mean values by weight group (Table 4). The U/S measurements, including saving scans to disk, were completed on each bird within two to three minutes. Dual-energy x-ray densitometry scans provided estimates of lean, fat and bone mass. As a check on DEXA scan accuracy, the gravimetric BW was compared to the total BW (DEXA) at scan time; in this study all 23 BW (DEXA) were within 2.0 % of gravimetric BW. As in the first experiment, lean tissue mass (DEXA) was accepted as a reliable indicator of whole bird protein. Immediately following DEXA scans, both left and right breast muscles were excised and the weights recorded. Total breast mass was

calculated by summation. The weights observed for breast mass, based on BW, were comparable to those observed in a male broiler study by Rabie and Szilágyi (1998).

Correlation was utilized to examine the relationships between U/S measurements, dissected breast mass, and lean tissue mass estimated by DEXA (Table 2). As in Experiment One, using mean values of matched U/S measurements, equaled or improved the correlation coefficient determined for a single-side measurement. The mean U/S measurements with the highest correlation to lean tissue mass (DEXA) and dissected breast mass were used in regression analysis (Table 3). Both linear and multiple regression were utilized, where U/S measurements were utilized as estimators of lean tissue mass (DEXA) and of dissected breast mass, with multiple regression improving the R^2 value from 0.86 to 0.92 and from 0.86 to 0.93 respectively. Therefore, it is reasonable to conclude that U/S measurements of the *Supracoracoideus* muscle may be used to estimate both breast mass and lean tissue mass in broiler breeder males. Additionally, this experiment shows that the U/S methodology may be used in vivo with minimal stress to the bird.

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Table 1. Mean body weights (by age) and body composition measurements determined by dual-energy x-ray absorptiometry (DEXA), ultrasound (U/S), and dissection in 10 age groups of broiler breeder females

Age (wk)	5	10	15	20	25	30	35	40	45	50
Number of birds	12	9	12	12	12	9	9	8	9	12
Gravimetric BW[1] (g)										
Target	620	1000	1520	2160	2950	3310	3587	3677	3768	3836
Farm	646	1007	1521	2171	2938	3337	3500	3684	3691	3842
Placement	576	923	1412	2034	2773	3127	3315	3477	3497	3661
Fasted	550	884	1348	1993	2800	2972	3171	3315	3332	3491
Pre-DEXA	549	879	1351	1994	2777	2959	3162	3291	3320	3470
Pre-U/S	551	880	1353	2000	2780	2962	3162	3294	3323	3476
DEXA (as-is)										
Lean (g)	474	797	1227	1677	2351	2518	2636	2643	2817	2843
Lean (%)	85.7	90.5	90.3	86.7	86.3	86.3	84.3	81.0	85.5	82.7
Fat (g)	68	65	101	212	312	335	418	556	403	518
Fat (%)	12.3	7.4	7.4	11.0	11.4	11.5	13.4	17.0	12.2	15.1
Bone (g)	1	18	31	47	63	65	74	64	75	76
Bone (%)	2.0	2.1	2.3	2.4	2.3	2.2	2.3	2.0	2.3	2.2
DEXA BW3 (g)	553	880	1359	1936	2726	2918	3128	3263	3295	3437
Ultrasound (mm)										
Superficial pectoralis muscle – VD[4]	4.8	6.2	8.2	11.1	10.8	11.5	10.8	11.8	10.7	11.8
Supracoracoideus muscle – VD	8.1	9.8	13.1	16.4	20.5	21.1	21.3	22.0	22.5	21.8
Supracoracoideus muscle – ML[5]	10.3	11.6	15.6	19.5	23.3	23.5	23.0	24.6	24.1	23.9
Abdominal fat pad	n/a	n/a	n/a	n/a	10.8	12.1	13.8	10.8	12.4	13.8
Dissection (g)										
Left breast	28.9	49.6	91.9	158.0	233.0	267.7	274.1	276.1	289.3	292.4
Right breast	29.2	51.6	93.1	157.8	229.0	265.3	268.3	277.2	276.7	286.4
Total breast	58.1	101.2	185.0	315.8	461.9	533.0	542.4	553.3	566.1	578.8
Abdominal fat	2.6	n/a	1.8	16.7	65.4	65.7	86.1	131.3	94.3	117.1

[1] Target = BW ±100 g used to select birds based on breeder guidelines; Farm = Mean BW at time of selection; Placement = Mean BW at time birds placed in cages for 72 h refeeding period before beginning of fast; Fasted = Mean BW at end of 44 h fasting period; Pre-DEXA = BW taken after thawing and before DEXA scans; Pre-U/S = BW taken after thawing and before U/S scans

[2] BW at time of selection not recorded by farm personnel, therefore Farm BW estimated based on linear regression equation Farm BW = 49.044 + 1.0424 * Placement BW), R2 = 0.9991, $P<0.0001$, N=50 where N represents those birds with both Farm BW and Placement BW recorded

[3] DEXA BW = BW as estimated by DEXA

[4] VD = ventral-dorsal measurement

[5] ML = medial-lateral measurement

Table 2. Ultrasound measurements correlated with lean tissue mass estimated by dual-energy x-ray absorptiometry (DEXA) and with dissected breast mass of broiler breeder females

Ultrasound measurements	Lean tissue mass (DEXA)		Total dissected breast mass	
Female	r-value	Observations	r-value	Observations
Right *Supracoracoideus* muscle – VD[1]	0.92	104	0.92	104
Left *Supracoracoideus* muscle – VD	0.93	103	0.93	103
Mean *Supracoracoideus* muscle – VD	0.95	103	0.95	103
Right *Supracoracoideus* muscle – ML[2]	0.93	104	0.92	104
Left *Supracoracoideus* muscle – ML	0.93	103	0.92	103
Mean *Supracoracoideus* muscle – ML	0.95	103	0.95	103
Male	r-value	Observations	r-value	Observations
Right *Supracoracoideus* muscle - VD	0.93	23	0.93	23
Left *Supracoracoideus* muscle – VD	0.88	23	0.88	23
Mean *Supracoracoideus* muscle – VD	0.93	23	0.93	23
Right *Supracoracoideus* muscle – ML	0.93	23	0.94	23
Left *Supracoracoideus* muscle - ML	0.93	23	0.94	23
Mean *Supracoracoideus* muscle - ML	0.94	23	0.96	23

[1] VD = ventral-dorsal measurement

[2] ML = medial-lateral measurement

P-value for every correlation was < 0.0001

Table 3: Estimating lean tissue and breast mass, using ultrasound (U/S) and dual-energy x-ray absorptiometry (DEXA), of broiler breeder females and males representing a range of body weights

To estimate:	Based on U/S measurements			ANOVA		
For Females	Intercept	Mean SCC-VD[1]	Mean SCC-ML[2]	DF	R^2	*P*-value
Total body lean tissue (g) / DEXA	- 969.86	+ 84.08	+ 72.90	102	0.94	< 0.0001
Total dissected breast mass (g)	- 299.69	+ 18.08	+ 18.46	102	0.94	< 0.0001
For Males	Intercept	Mean SCC-VD	Mean SCC-ML	DF	R^2	*P*-value
Total body lean tissue (g) / DEXA	- 1576.58	+ 116.31	+ 106.05	22	0.92	< 0.0001
Total dissected breast mass (g)	- 572.73	+ 25.08	+ 29.87	22	0.93	< 0.0001

To estimate:	Based on DEXA measurements		ANOVA		
For Females	Intercept	Lean tissue mass (g)	DF	R^2	*P*-value
Total dissected breast mass (g)	- 71.09	+ 0.23	103	0.98	< 0.0001
For Males	Intercept	Lean tissue mass (g)	DF	R^2	*P*-value
Total dissected breast mass (g)	- 156.56	+ 0.25	22	0.97	< 0.0001

[1] SCC-VD = *Supracoracoideus* muscle, ventral-dorsal measurement
[2] SCC-ML = *Supracoracoideus* muscle, medial-lateral measurement

Table 4: Body composition (BC) estimated by dual-energy x-ray absorptiometry (DEXA), ultrasound (U/S) measurements, and dissected breast mass observed for broiler breeder males in three body weight classes

	Weight Class (Number)					
	Lightweight (5)		Middleweight (5)		Heavyweight (13)	
	x	sd	x	sd	x	sd
Gravimetric BW (g)						
Pre-DEXA (thawed)	1413.2	378.1	3250.8	334.5	4721.4	475.0
Pre-U/S (live)	1428.0	382.1	3278.4	342.6	4732.7	477.6
DEXA (as-is)						
Lean (g)	1282.2	342.8	2968.3	368.8	4341.9	474.0
Lean (%)	90.1	2.9	91.8	2.1	92.3	2.1
Fat (g)	117.2	35.5	216.0	53.9	279.8	104.8
Fat (%)	8.5	2.8	6.8	1.9	5.9	2.0
Bone (g)	19.2	4.9	47.4	7.4	81.3	10.3
BW(g) estimated by DEXA	1418.6	358.8	3231.7	358.9	4703.0	499.1
Ultrasound (mm)						
Left PEC[1] – VD[2]	5.46	1.36	8.16	1.44	13.09	0.98
Right PEC – VD	5.94	2.17	9.30	1.64	10.65	1.60
Mean PEC – VD	5.70	1.64	8.73	1.35	11.87	0.92
Left SCC[3] – VD	10.86	0.86	16.80	2.17	21.71	3.38
Right SCC – VD	11.26	1.67	17.38	1.58	20.34	2.22
Mean SCC – VD	11.06	1.26	17.09	1.69	21.02	2.50
Left SCC – ML[4]	15.78	3.52	25.12	3.11	31.28	3.35
Right SCC – ML	15.74	3.25	25.24	2.86	32.68	3.85
Mean SCC – ML	15.76	3.36	25.18	2.86	31.98	3.10
Dissection (g)						
Left breast mass	88.36	30.72	270.24	39.64	476.81	49.05
Right breast mass	80.67	32.59	266.04	43.55	463.01	69.48
Total breast mass	169.03	62.87	536.28	79.82	939.82	111.83

[1] PEC = *Superficial pectoralis* muscle
[2] VD = ventral-dorsal measurement
[3] SCC = *Supracoracoideus* muscle
[4] ML = medial-lateral measurement

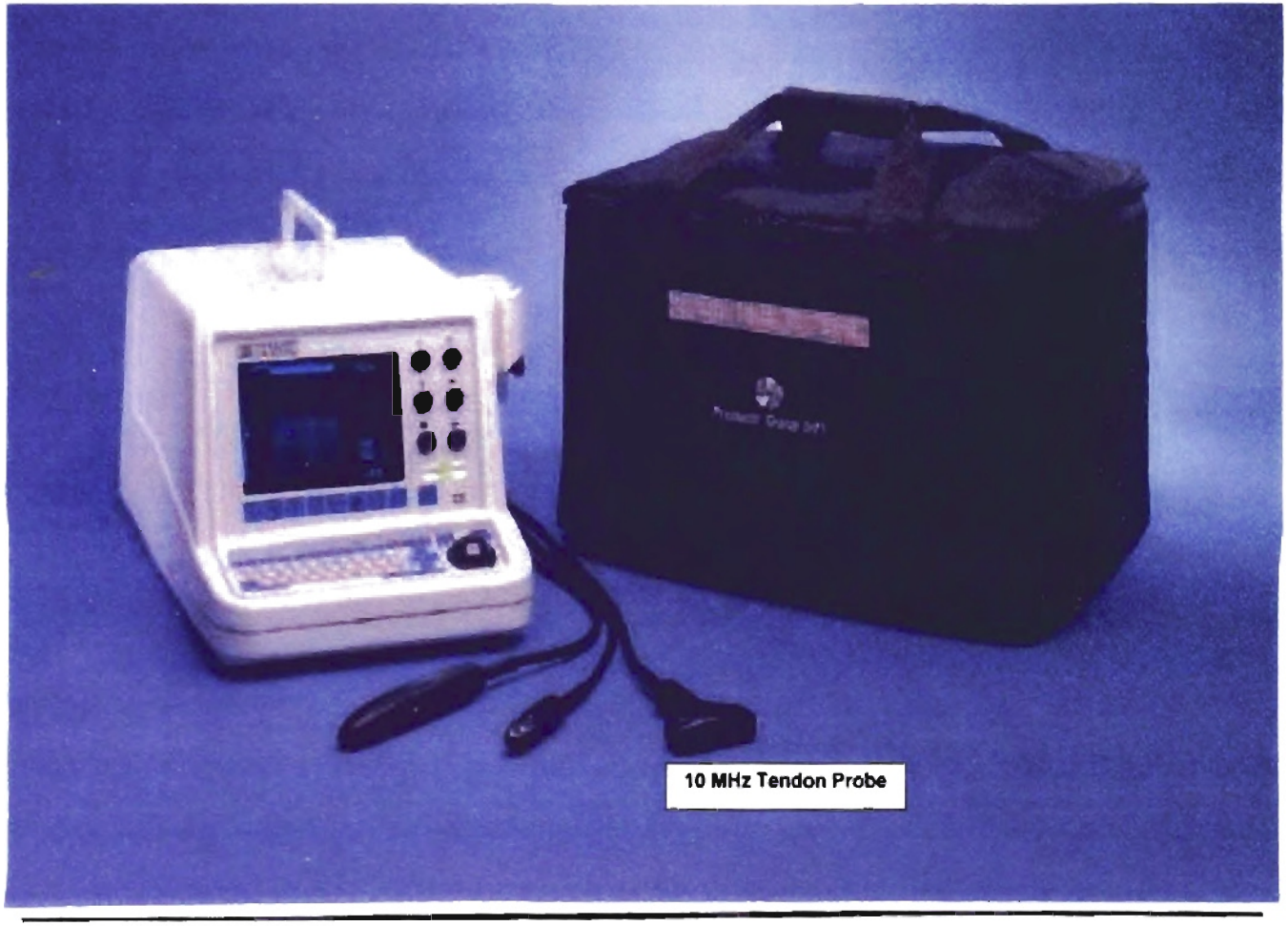
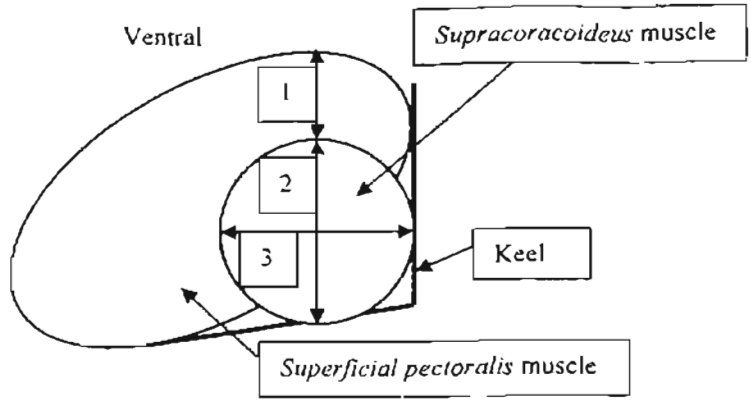


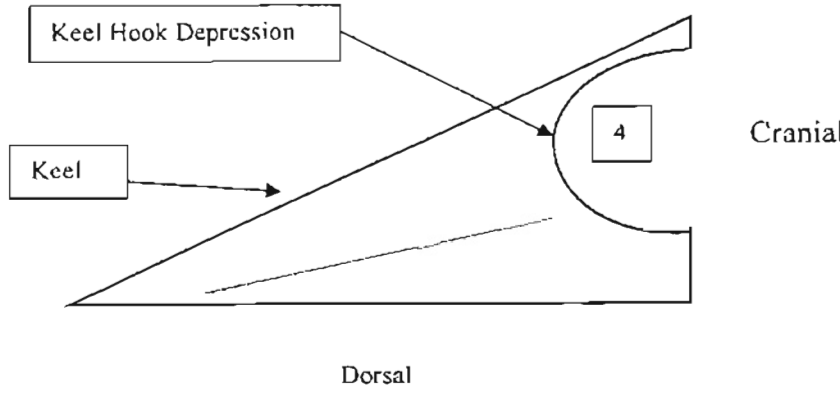
Figure 1. Ultrasound system (Dynamic Imaging Concept MLV Veterinary Ultrasound Scanning System) used to scan both abdominal fat and breast muscle in broiler breeder females and breast muscle in broiler breeder males. The 10 MHz tendon probe was used for all scans.

Breast Muscle Measurments

1 - *Superficial pectoralis* muscle thickness (ventral-dorsal)
#2 - Depth (ventral-dorsal) of *Supracoracoideus* muscle parallel to the ventral extension of the keel
3 – Width (lateral-medial) of *Supracoracoideus* muscle at right angles to depth measurement



All measurements of breast muscle taken immediately cranial to the keel hook depression (# 4)



Abdominal Fat Pad Measurements

1, # 2 & # 3 – locations and order of fat pad thickness measurements

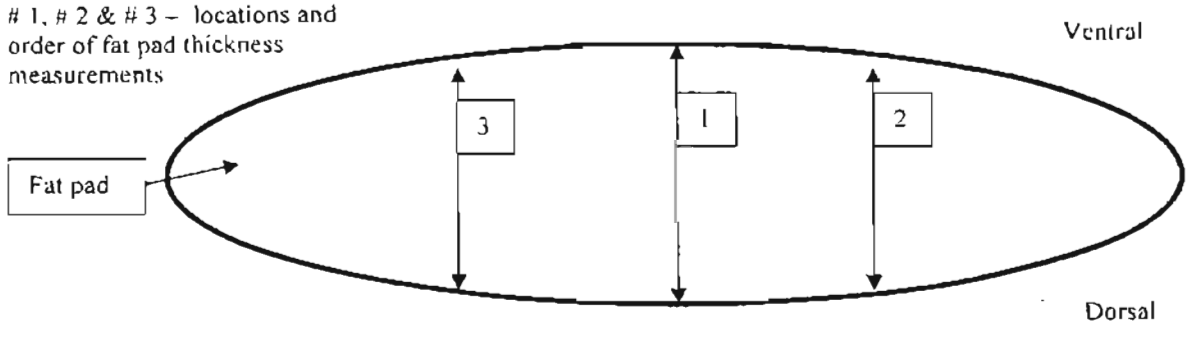


Figure 2: Schematics of ultrasound measurements made of breast muscle in broiler breeder females and males and of abdominal fat pad thickness in broiler breeder females.

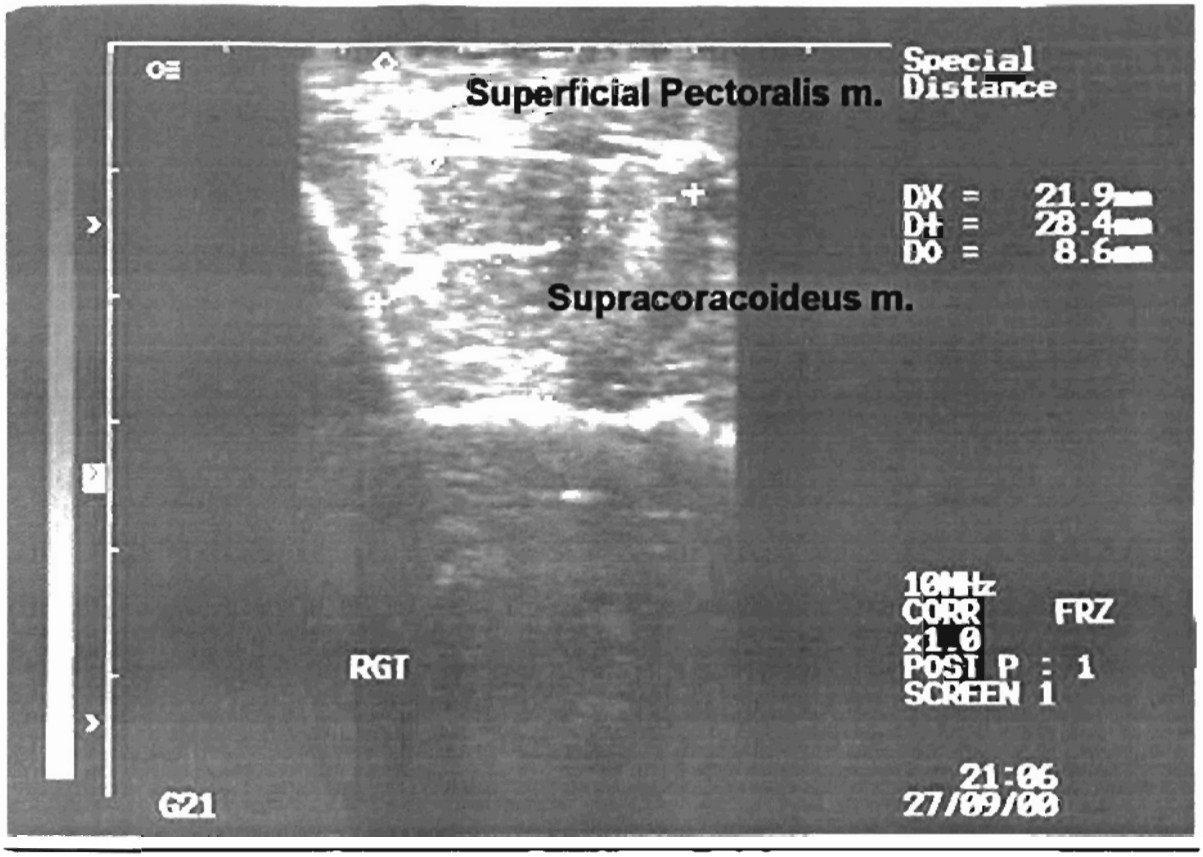


Figure 3. Ultrasound (U/S) scan of right (RGT) breast muscle (m.) demonstrating how the three measurements were marked by 'x', '+' and '◊' with the distance between each pair of symbols (DX, D+ and D◊) calculated by the U/S software.

CHAPTER VII

SUMMARY

Research has demonstrated the existence of a negative relationship between body weight and reproductive efficiency in both male and female broiler breeders, i.e. as body weight increased, reproductive efficiency decreased. Additionally, when broiler breeders reach sexual maturity is not only related to body weight and age, but to body composition. Therefore being able to accurately assess body composition is important information that can be used by the broiler breeder industry.

Since heat is a by-product of the metabolic process, heat production is one method of examining the metabolic processes that are the basis for growth, i.e. tissue accretion. Defining the relationships that exist between heat production and tissue accretion components (i.e. body composition) as well as egg production was the focus of the first study presented in this dissertation. Heat production in broiler breeder females, representing a range of ages (5 to 50 weeks) and body weights (646 to 3842 g), was examined in fed and fasted birds housed in three ambient temperatures during both dark and lighted photoperiods. In all cases, fasted heat production was lower than fed heat production ($P<0.05$), heat production during the lighted photoperiod was higher than during the dark photoperiod ($P<0.05$), and as body weight increased the heat production per unit body weight (kJ/hr/kg body weight) decreased ($P<0.05$). However, differences between heat production for the different ambient temperatures were less well-defined with the additional unexpected finding that the heat production data, specifically basal

metabolic rate data, indicated that the warmest ambient temperature ($32^0 - 1^0$ C) represented the thermoneutral zone for all age groups. Since this temperature zone was considerably higher than expected (18^0 to 21^0 C), a review was made of the data with no change in the results. However, when it was taken into consideration that this study was performed during the summer months and that the environment from which the birds were selected was warm to hot, the conclusion reached was that the birds were acclimated to high ambient temperatures; this conclusion was supported by previous reports by other researchers.

An examination of the relationship of heat production to body composition, as determined by proximate analysis and dual-energy x-ray absorptiometry, resulted in the development of regression equations that allow moderately good estimations (R^2 ranging from 0.6760 to 0.8091) of heat production based on the body components determined by both methods. Additionally, the correlations between like body components as determined with proximate analysis, the gold standard for determining body composition, and with dual-energy x-ray absorptiometry were very good ($r>0.9552$). The relationship between heat production and body weight was further examined by determination of the metabolic body size. This method examines the linear relationship between heat production, specifically basal metabolic rate, and body weight. Data in this study provided an estimate for metabolic body size of 0.67, which agrees with classical calculations for birds.

Finally, an examination was made of the difference in heat production between those sexually mature broiler breeder females that laid an egg and those that did not lay an egg during a given time period. This examination revealed a significant difference

($P \leq 0.05$) in the heat production between the two groups. Using regression analysis, an equation for estimating heat production in non-laying birds was developed and used to predict what the heat production in the birds that laid eggs would be during a non-laying period. This allowed estimation of heat production due to oviposition.

A second heat production study, this time of broiler breeder males representing a range of body weights (0.9 to 5.0 kg), examined fasted heat production during a lighted and a dark photoperiod. As seen with the female broiler breeders, a decrease was observed in heat production per unit weight as overall body weight increased. An increase in heat production was observed as birds moved from a dark environment to a lighted environment ($P<0.05$). Regression equations were developed for estimating heat production both on a per bird basis and on a per unit body weight basis (R^2 ranging from 0.81 to 0.95). A determination of metabolic body size was also made, with the resultant exponent of 0.66 almost matching the classical exponent of 0.67.

Two additional studies examined the relationship between several methods of determining body composition. The first examined body composition in broiler breeder females using several morphological methods of measurement (girth, shank length, and skin-fat pad thickness), dissected abdominal fat weight, dual-energy x-ray absorptiometry estimates, proximate analysis determinations, and carbon-nitrogen tissue content determinations. Additionally, whole bird energy was determined by bomb calorimetry. The relationships of the morphological measurements to dual-energy x-ray absorptiometry estimates and dissected abdominal fat weight were determined. The best correlation found was between girth and the dual-energy x-ray absorptiometry estimate of lean tissue ($r=0.94$) followed by the correlations of the skin-fat pad thickness to dissected

abdominal fat weight (r= 0.81) and the dual-energy x-ray absorptiometry estimate of fat mass (r=0.86). Equations for estimating lean mass based on girth measurements and for estimating fat mass based on skin-fat pad thickness were developed.

An examination of the relationship among the remaining three methodologies, proximate analysis, dual-energy x-ray absorptiometry, and carbon-nitrogen tissue content, revealed good correlations ($r>0.95$) between the methodologies for each type of body component. Equations for interrelating these three methodologies were developed ($R^2 \geq 0.90$). Additionally, an equation estimating whole bird energy based on the carbon-nitrogen tissue content was developed ($R^2=0.88$).

The final study examined body composition in both male and female as estimated using dual-energy x-ray absorptiometry and ultrasound. Additionally, breast weight and abdominal fat weight in the females and breast weight in the males were determined from dissection. Since dual-energy x-ray absorptiometry had been previously shown to provide an accurate estimation of body composition when compared to proximate analysis, it was used as the standard in this study. In the females, cross-sectional ultrasonic measurements were made of breast muscle and of the abdominal fat pad. These measurements, when compared to dual-energy x-ray absorptiometry estimates for lean and fat respectively, revealed a strong correlation for the breast measurement to lean estimation ($r \geq 0.92$) but a poor correlation for the abdominal fat pad to total body fat estimation ($r=0.22$). Due to the poor abdominal fat pad to total body fat correlation found with the female data, only the breast measurements were made with ultrasound during the male broiler breeder segment of the study. As with the females, a strong correlation ($r \geq 0.88$) was found in the males between the ultrasonic breast measurements and lean

tissue mass estimated by dual-energy x-ray absorptiometry. A strong correlative relationship was also present for both males and females between dissected breast weight and ultrasonic breast measurements ($r \geq 0.88$ in males, $r \geq 0.92$ in females). The best correlated components were then examined using multiple regression to develop predictive equations for estimating total body lean mass based on ultrasonic measurements, and for estimating breast mass based on dual-energy x-ray absorptiometry estimates of lean tissue and on ultrasonic breast measurements.

The studies described herein have demonstrated that the relationships between heat production and various methodologies for determining body composition may be reliably used as the basis for estimating heat production based on body composition. Additionally, whole bird energy was found to be closely correlated to carbon-nitrogen tissue content with a resultant equation developed to estimate whole bird energy. Dual-energy x-ray absorptiometry estimates of body composition were found to be very accurate when compared to the classical body composition determination method of proximate analysis. This knowledge allows researchers to confidently use dual-energy x-ray absorptiometry estimates of body composition as a basis for other determinations. Body composition determination methods that can be used in field conditions, girth, skin-fat pad thickness, and ultrasound, were determined to be good estimators of lean mass, fat mass and lean mass, respectively, when compared to dual-energy x-ray absorptiometry estimates. Additionally, ultrasound may be used to estimate breast mass, as demonstrated by its correlative relationship to dissected breast mass. Overall, these studies have provided evidence to support the use of the various body composition determination methodologies in broiler breeder males and females.

VITA

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Master of Science

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