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Portal-Drained Viscera, Hepatic and Lower Hindquarter Net Flux of Nitrogen and Energy Metabolites in Ruminants Fed Protein Supplements Containing Increasing Amounts of Bound Nitrogen and Structural Carbohydrates

Byron Clark Housewright
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Byron Clark Housewright entitled "Portal-Drained Viscera, Hepatic and Lower Hindquarter Net Flux of Nitrogen and Energy Metabolites in Ruminants Fed Protein Supplements Containing Increasing Amounts of Bound Nitrogen and Structural Carbohydrates." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

John C. Waller, Major Professor

We have read this thesis and recommend its acceptance:

Richard N. Heitman, James D. Quigley III

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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PORTAL-DRAINED VISCERA, HEPATIC AND LOWER HINDQUARTER NET FLUX OF NITROGEN AND ENERGY METABOLITES IN RUMINANTS FED PROTEIN SUPPLEMENTS CONTAINING INCREASING AMOUNTS OF BOUND NITROGEN AND STRUCTURAL CARBOHYDRATES

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ABSTRACT

Fifteen experiments were conducted on nine mature, nonlactating, nonpregnant ewes to compare corn distillers dried grains with solubles (DDGS) to soybean meal (SBM) on both an energy and a protein basis. Ewes were equipped with chronic indwelling catheters in the caudal aorta and caudal vena cava, portal, hepatic and mesenteric veins. Ewes were assigned randomly to one of three isocaloric, isonitrogenous diets. Diets were corn cob based and balanced for energy with the addition of corn starch. Protein source for these diets was either SBM, DDGS or a combination of the two, mixed so that 50% of the protein was supplied by DDGS and the remaining 50% supplied by SBM (DDGS + SBM). Animals were fed once daily and consumed a minimum of 1.1 kg/d for a minimum of 10 days prior to blood collection periods. Paraaminohippurate (PAH) was infused into a mesenteric vein and 1 h was allowed for near equilibration. Subsequently, six samples were taken simultaneously from caudal aorta, caudal vena cava, hepatic and portal veins, at 30 min intervals. Following the sixth sample the infusion line was changed to the caudal aorta. A 15 min equilibration time was followed by three samples taken at 15 min intervals, simultaneously from the caudal vena cava and a jugular vein. These samples were used to calculate blood flows across the hindquarters. Samples underwent sameday analysis for glucose, free fatty acids (FFA), PAH (for blood flow), beta-hydroxybutyrate (BOHB) and acetoacetate (ACAC). Plasma was harvested and frozen for later analyses of VFA, ammonia nitrogen (AN), blood urea nitrogen (BUN) and alpha-amino N. Portal drained viscera (PDV) values in kcal/d were similar across

diets, indicating similar energy availability from each of the three diets. FFA arterial concentrations were elevated (P<.01) when ewes received DDGS + SBM as a result of a 4 - 5 fold increase (P<.01) in hindquarter release of FFA. No physiologically significant changes occurred with respect to either ACAC or BOHB. A decrease in the percentage of energy released into the portal blood in the form of VFA was noted in DDGS and DDGS + SBM vs. SBM. PDV release of AN was less (P<.05) on DDGS (8.7 um/h) than SBM (13.8 um/h), while DDGS + SBM was not significantly different than either treatment. Release of BUN into the portal blood was higher (P<.1) on DDGS than SBM, however, liver uptake and calculated TSP release of BUN was similar across diets. Gut release of alpha-amino N was similar across treatments (SBM 8.4 um/h, DDGS 8.4 um/h, DDGS + SBM 13.2 um/h). Based on data reported in this study it seems that DDGS is at least equal to SBM as a protein supplement, in terms of energy and nitrogen release by the gut and subsequent hepatic and peripheral tissue metabolism.

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INTRODUCTION

Corn distillers dried grains with solubles (DDGS) has received a great deal of attention throughout the last 40 years as a viable byproduct feedstuff. DDGS is a byproduct of the ethanol distilling industry. Through the processing of corn grain much of the starch is removed, and the remaining product is the result of a conversion of a once high energy feed to one that is considered as a protein supplement containing approximately 28 - 30% protein. The bulk of the research conducted on DDGS has revolved around the amount of undegradable intake protein (UIP) contained in the feed. Protein escaping rumen degradation can be an advantage to a feedstuff, although this is dependent on the amino acid profile of the feed. A high quality protein feed that contains large amounts of UIP shows advantages through its ability to deliver an amino acid profile to the blood essentially identical to that which is fed. A feed containing little UIP is quickly degraded in the rumen and may be converted to microbial protein leading to problems such as, only a fixed quantity is produced and wastage. Therefore, because of the comparatively large amounts of UIP contained in DDGS it would seem to have immediate advantages over many other protein sources.

However, the same process that converts this grain into a high protein source has raised concerns about the availability of DDGS in terms of energy and as a protein source. Heating of the product has been proven to increase ADF and NDF content as well as increasing nitrogen that is bound as acid detergent insoluble nitrogen (ADIN), and thereby rendering it unavailable for use by the animal.

The use of multicatheterization allows for in vivo isolation of tissue beds, i.e., hepatic and hindquarter tissue. These tissues are still under direct hormonal control and subject to changes brought on by other physiological factors. Multisite sampling is valuable in the recognition of metabolic processes that transcend tissues.

LITERATURE REVIEW

Availability of ADIN in Feedstuffs

Distillers Grains as a Byproduct Feed

Corn distillers dried grains with solubles (DDGS) have been evaluated as a potential protein supplement for the past forty years. It has been well established that corn protein is slowly degraded in the rumen and thereby causing much of it to escape the rumen for degradation in the abomasum and small intestine (Klopfenstein, 1985). Furthermore, it has been identified that heating undoubtedly increases the rumen undegradable intake protein (UIP) quantities contained in DDGS (Klopfenstein et al., 1976) with bypass increases more prevalent in the condensed solubles portion (Klopfenstein et al., 1981). Because of type of protein contained in corn and processing procedures use in production of DDGS. Distillers grains are an excellent representative feed to assist in the identification of availability of bound nitrogen.

ADIN Usage in Ruminants

Relationship between heating and increases in ADIN has been researched more heavily in forages (Britton, 1986). Many researchers have identified that heating increases ADF content of forages as well as ADIN values (Goering et al., 1973, 1974, 1975; Yu and Thomas, 1975; Thomas, 1976a, 1976b; Yu and Viera, 1977; Yu et al., 1977; Yu, 1976, 1977). Increases in ADF content of forages could possibly be an artifact of the procedure or increases in ADIN (Thomas, 1982).

Thomas et al. (1982) reported a study involving six growing Holstein females, growth response and blood parameter response when receiving haylage with ADIN values of 48 to 51%. They noted a decreased growth from .9 kg/d to .4 kg/d with feeding of high ADIN haylage. Also, blood urea nitrogen was reduced by 50% from pretrial levels. Thomas et al. (1982) cautions that heating would be expected to have a highly correlated relation to ADIN level, but a regression developed for specific conditions can not be expected to be applicable in all cases.

Relation of ADIN to Heat Treatment

Researchers have shown a relation of ADIN content to both degree of heat exposure and length of exposure to heat. Yu (1976) used the term degree hours above a given temperature to quantify exposure to heat treatment and demonstrated that these were reasonable units to express heat exposure. Thomas (1976a) using units of degree days above a given temperature to quantify heat treatment noted correlation coefficients of .72 to .94 for ADIN vs. degree days above 35 degrees. He went further to state that under laboratory conditions the rate of formation of ADIN increased as temperature was increased.

Goering et al. (1972, 1973) and Yu (1977) concluded that grasses experienced more rapid formation of ADIN than did legumes under the same conditions. These researchers noted a difference in digestible nitrogen than what was expected, based on ADIN content. Therefore, these scientists reported that some chemical differences in ADIN formed in legumes vs. that which was formed in grasses. Because of this conclusion,

questions remain as to the applicability these forage studies to the feeding of higher protein feeds such as DDGS.

Quantifying Available Bound Nitrogen

Thomas (1982) indicated that calculation of "available protein" is done by subtracting ADIN from total nitrogen and then multiplication by a factor of 6.25. He did state that at publication time little was known about the relation of forage ADIN to other feeds, but in discussion following the Thomas (1982) presentation he was asked about differences in ADIN contained in DDGS or Brewers' grains with those found in forages, he states "Van Soest has tested this with protease. His feeling is that it is indigestible."

In an attempt to quantify nitrogen utilization when byproduct feedstuffs are fed, Britton et al. (1986) conducted three feeding trials using DDGS and other protein supplements. Recovery of ADIN and pepsin insoluble nitrogen (PIN) in feces and the relation of nitrogen digestibility to feed content of ADIN and PIN was investigated. In one of the three trials additional heating, regardless of pH, did not affect apparent nitrogen digestibility. When DDGS was compared to soybean meal (SBM), apparent nitrogen digestibility was statistically similar even with additional heat treatments of DDGS. Furthermore, digestion trials conducted by Britton et al. (1986) revealed that heat treatments of DDGS did not effect digestible nitrogen content of the feedstuff, however, analysis of feed by ADIN and pepsin insoluble nitrogen (PIN) indicated that there was in fact additional protein damage by heating. Questions were raised as to the validity of the ADIN measurements. Recovery of ADIN

was very low in treatments where high ADIN feeds were fed and greater than 100% in low ADIN feeds. Therefore, the assay may not measure a quanitatively recoverable indigestible nitrogen fraction in the feces.

Evaluation of Amino Acid Profiles of UIP

Protein supplements considered to be high in rumen undegradable intake proteing (UIP) have been extensively researched. According to Muscato (1983), the majority of data related to UIP values has dwelled on duodenal values for total amino acid nitrogen. Because of increased interest in the field of UIP, formulation of diets and research must address the amount or proportion of individual amino acids available for absorption in the small intestine. Cereal grains have been found to be deficient in lysine as compared to animal requirement, further processing of cereal grains such as corn grain to DDGS through heating and starch extraction could possibly limit lysine availability even more (Bjarnson and Carpenter, 1970).

Maillard Reaction and Amino Acid Availability

Mechanism of Maillard Reaction

Increased lysine binding mentioned previously (Bjarnson and Carpenter, 1970) could be attributed to the formation of Maillard products during the processing of DDGS. The Maillard reaction as described by Adrian (1974) is the reaction involving an aldehyde or ketone and an amino acid. This reaction between an aldehyde or ketone and an amino acid first gives an addition compound followed by a

nitrogen-substituted glucosamine. Following an Amadori rearrangement a 1-amino 1-deoxy 2-ketose type molecule is formed. Researchers have noted that occurence of the Maillard reaction is observable at room temperatue but occurs with greater velocity at increased temperatures (Maillard, 1912; Lea and Hannan, 1949; Overby et al., 1959). Additionally pH may increase during the occurence of the Maillard reaction. Lea (1950) reported that formation of Maillard products increased linearly with increased alkalinity from pH 3 to at least pH 8 and possibly continuing in a linear fashion to pH 10.

Amino Acid Availability

Adrian (1974) noted the first compounds formed in the Maillard reaction cannot be broken enzymatically; therefore, from a nutritional standpoint the amino acids are lost at the first step of the Maillard reaction. These enzyme resistant properties were examined by Hagan et al. (1970), who discovered the quantity of serum glycine in rats was only slightly elevated From controls by the addition of fructose-glycine, as opposed to values obtained when rats were supplemented with free glycine. Free amino acid reactivity was found to be directly related to the distance between the alpha-carbon and the amino groups, this is seen in the reactivity of lysine (Lento et al., 1958). In the case of lysine, the amine group is located on the epsilon-carbon, putting considerable distance between the alpha-carbon and amine group. Thus, lysine is 6 times more reactive than norleucine, which only has amino group in the alpha position (Lento et al., 1958; Underwood et al., 1959).

Susceptibility of Amino Acids

With the exception of tryptophan, all free amino acids react similarly, for example the greater the acidity of the medium the greater their stability and their reactivity begins at pH 7 and increases with velocity as medium increases in alkalinity (Adrian, 1963). Adrian (1963) also found tryptophan exhibited almost the reverse activity, when compared to the other free amino acids, as tryptophan becomes more active as the medium increases in acidity. Identification of free amino acid loss is accomplished by recognition of specific compounds formed by each amino acid in the Maillard reaction. Additionally, the premelandoin products formed are more indicative of the amino acid than the sugar involved in the reaction (Adrian, 1974). Amino acid reactivity changes somewhat when they are bound in a protein chain. The amino acid most susceptible to destruction by the Maillard reaction is at the amino terminus, following by basic amino acids (especially lysine) (Adrian, 1974). It was found destruction of lysine occurs 5-15 times more often than other amino acids. Sulfur containing amino acids are also sensitive to Maillard reaction, usually reacting after lysine (Adrian, 1974). Tryptophan very often is subject to Maillard changes along with the sulfur amino acids. Evidence presented in reference to the Maillard reaction and its known presence in DDGS reinforces the need for research in the area of available nitrogen from DDGS.

Energy Availability of Corn Byproduct Feeds

Energy Availability of Corn Bran

An area of relative research inactivity is of energy availability of byproduct feeds. High fiber content has been long considered a hinderance to digestibility of a feedstuff. One exception to this "rule" is corn fiber or corn bran. According to Klopfenstein (1985) DDGS contains approximately 35% fiber compared to 12% in corn grain. DeHaan et al. (1983) found that corn bran is highly digestible in ruminants, (87%). Further digested at a very rapid rate (6.2 h). A comparison of corn bran to corn grain as an energy supplement to high forage rations was reported by Klopfenstein et al. (1985). This comparison indicated calves increased intakes and gains when 25 or 50% of the corn cob alfalfa diet was replaced by either corn bran or corn grain. However, it was also noted calves had increased intakes, and were 5% more efficient in feed utilization when receiving corn bran over those receiving corn grain. Additional work by Klopfenstein et al. (1985) provided information on supplementation of brome hay with corn bran or corn grain. An increase in digestibility of brome grass was observed at 25% replacement with corn bran, but no additional increase in digestibility was seen by increasing percentage of corn bran over 25%. Researchers concluded that corn bran was at least equal to and in some cases greater in feed quality to corn grain.

Negative Associative Effects

A major problem related to supplementation of a high roughage diet with an ingredient high in energy is one commonly termed as negative

associative effect. Negative associative effect can be attributed to a number of causes: 1) lowering of ruminal pH (Stewart, 1977; McDonnell et al., 1979; Mould et al., 1983), 2) increased rate of passage (McDonnell et al., 1979; van der Linden et al., 1984), 3) decreased potential for fiber digestion (Miller and Muntifering, 1985), 4) increased lag time of fiber digestion (Mertens and Loften, 1980), or 5) decreased cellulolytic bacteria population in the rumen (Henning et al., 1980). Oliveros et al. (1989) stated that supplementation of high roughage diets with high fiber energy supplements could diminish the occurrence of negative associative effects. DeHaan (1983) found corn bran to contain approximately 70% NDF, but as stated previously, this research reported in vitro disappearance of corn bran NDF to be about 87%. Oliveros et al. (1989) supplemeted sheep and cattle to study negative associative effects in diets supplemented with by corn gluten feed, corn bran and corn grain. Researchers concluded that corn bran was equal to corn grain as an energy supplement. Also fewer animals are likely to suffer from the problem of negative associative effects, thereby improving gains and feed efficiency.

Multicatheterization Applications

Evaluation of Complete Metabolic Pathways

Understanding mechanisms of action in metabolism of nutrient compounds as well as hormones is important in interpretation of physiological responses to different nutritional stimuli. In vivo isolation of tissue beds allows for measurements of metabolic activity

thoughout a metabolic pathway. As defined by Newsholme and Leech (1983) a metabolic pathway is "a series of enzyme-catlysed reactions, initiated by a flux-generating step and ending with either the loss of products to the environment, to a stored product (that metabolic 'sink') or in a reaction that precedes another flux-generating step..." They also stated that a metabolic pathway transcends tissues; therefore, glycolysis from glucose is not a complete metabolic pathway, but begins with glycogen in the liver. This definition of a metabolic pathway illustrates the necessity for measurements to encompass all tissue beds that are involved in a complete metabolic pathway. Therefore, multisite sampling is mandatory in elucidating information dealing with metabolic pathways.

Single Site Sampling

Single site sampling has merits in limited situations, for example, a sample taken from the jugular vein that is evaluated for non-esterified fatty acids can help to obtain information about physiological status of an individual. Single site samples are only indicative of a given concentration at a specific time, which in many cases can be inconclusive when comparing treatment values. These single site concentrations do not reflect a shift in site of production or uptake of a specific metabolite, i.e. the alimentary or hepatic production of ketone bodies. The concentration of a metabolite is a function of its rate of appearance or de novo synthesis, or disappearance and subsequent excretion through fecal, urinary or gaseous excretion. This appearance or disappearance can be accomplished through absorption by the gastrointestinal tract into portal blood, following which, the metabolite can be acted upon by hepatic

or peripheral tissues. However, in evaluating metabolic changes occurring under different conditions, it is necessary to assay through the progression of metabolic pathways at separate sites in the body.

Catheter Placement and Type of Information Received

Catheterization of splanchnic and hindquarter blood vessels is beginning to find a prominant place in ruminant nutrition research. First described in detail by Katz and Bergman (1969), catheterization has documented specific metabolism rates of hormones, energy metabolites, amino acids and other nitrogeous compounds.

Recently the technique has been altered by Zanzalari et al. (1989), placement of catheters remain as those originally published by Katz and Bergman (1969), only technique in placement of the catheters was altered. Catheter placement in the caudal aorta, caudal vena cava, portal, hepatic and mesenteric veins allowed for calculation of venoarterial differences, which are indicative of specific tissue uptake and/or utilization, or de novo production or synthesis of a metabolite. Arterial concentrations greater than venous measurements demonstrate uptake of a metabolite by the isolated tissue bed. On the other hand, arterial concentrations lower than that of veinous measurement indicate synthesis of a metabolite or hormone (Heitmann, 1989). Multisite sampling in this way is superior to single site sampling in that flux rates of a metabolite can be calculated when venoarterial differences are used in combination with blood flow rates. However, venoarterial differences reported without blood flow rates should be viewed with some reservation. This need for blood flow rates was illustrated by Heitmann and Fernandez (1986). These researchers

reported venoarterial differences indicating increased hepatic uptake of free fatty acids by insulin-treated diabetic sheep, compared to diabetic sheep not treated withe insulin; however, there was a concomitant decrease in portal and hepatic blood flow rates which resulted in similar flux rates of free fatty acids for both treatment groups. The combination of venoarterial differences with blood flow rates results in net flux rates across specific tissue beds.

Calculation of Net Flux Rates

Flux rates for portal drained viscera, hepatic, total splanchnic, renal and hindquarter may be calculated by the following equations:

Portal drained viscera = $PF \times (C_p - C_a)$

Hepatic = $PF \times (C_h - C_p) + AF \times (C_h - C_a)$

Total splanchnic = $HF \times (C_h - C_a)$

Renal = $RF \times (C_r - C_a)$

Hindquarter = $VF \times (C_v - C_a)$

where PF, AF, HF, RF, and VF are whole blood flow or plasma flow rates for the portal vein, hepatic artery, hepatic vein, renal vein and femoral vein, respectively. Specific metabolite concentrations in whole blood or plasma, in the portal vein, femoral artery, hepatic vein, renal vein and femoral vein are noted as C_p, C_a, C_h, C_r and C_v, respectively.

In calculation of flux rates, a positive number indicates production (release) of a metabolite, while a negative flux rate is due to uptake (removal) of the measured metabolite. Samples from muliticatheterized animal are extremely sensitive to sampling techniques.

Mechanics of Blood Collection

Care when removing samples from catheters is of extreme importance. Samples are taken simultaneously from all sample catheters to minimize error. Mechanics of blood collection from catheters was discussed by Bergman (1975), who observed that collections must be made slowly and with minimal pressure to avoid laminar blood flow to tissues. Occurence of laminar flow is of special concern when sampling portal vein blood.

Application of Extraction Ratios

In order to assist the investigator as to the reason for increased or decreased uptake of a metabolite. Additional calculations can be made that represent the percentage of the metabolite presented to a tissue that is taken up by that tissue, these are termed as extraction ratios. Extraction ratios that change significantly when different treatments are administered can be partially attributed to some physiological mechanism. Extraction ratios can be used to determine if uptake of a metabolite is passive and due solely to circulating concentrations, in this case a change in net flux will be seen but extraction ratios will remain constant. Because the disappearance of a metabolite is a function of the amount of that metabolite presented to the tissue, this amount can be changed by either an increase in circulating concentrations or a change in blood flow rates. Through the calculation of extraction ratios both of these factors are taken into consideration to arrive at a percentage of the metabolite that is removed by a tissue.

Unidirectional Flux Rates

Using net flux techniques with infusion of radioactive isotopes allows calculation of unidirectional flux rates. By using radioactivity, information can be gained about intraorgan metabolism. Net flux is a function of a metabolite's uptake and utilization and simultaneous de novo production and release. An increase in net flux could be due to a decrease in its irreversible utilization or an increase in its de novo synthesis. By using radioactive labelling, molecule tracing can determine utilization and/or de novo synthesis (Heitmann, 1989). Various applications of the multicatheterization technique have been used by researchers. However, the theme of the research remains the same regardless of the various conditions of the animal or the treatments imposed. This technique measures the flux of any hormone or metabolite across any tissue bed that can be isolated in vivo. The bulk of available research is in area of ketone body flux across alimentary and hepatic tissue in animals at various physiological states.

Applications of Multicatheterization

Evaluation of Ketone Bodies

Ketone bodies have been the most studied metabolites using the multicatheterization technique and clearly demonstrate the advantages of multisite sampling. Many have described the utilization of ketone bodies by hindquarters and kidney as indicated by lower concentrations in renal and femoral veins than arterial concentrations (Heitmann et al., 1986; Heitmann et al., 1987; Kaufman and Bergman, 1971; Eisemann et al., 1988).

Additionally, concentrations of BOHB in hepatic veins was greater than arterial concentrations because of production of BOHB by conversion of free fatty acids and acetoacetate (Katz and Bergman, 1969; Brockman, 1976; Heitmann et al., 1986). Pell and Bergman (1983) described metabolism of beta-hydroxybutyrate (BOHB) by cephalic tissue. These researchers observed arterial concentrations were similar to those in jugular blood, demonstrating the ruminant brain does not use BOHB.

Multicatheter sampling of fed and progressively fasted ruminants by Heitmann and coworkers (1986), reported lower arterial concentrations of BOHB than those noted in corresponding portal vein samples, indicating alimentary ketogenesis and release of ketones by the gut into portal blood. However, in three day fasted animals portal vein concentrations were lower than arterial concentrations, indicating alimentary use of ketone bodies as an alternative energy source by the gut. These researchers also reported, in fasted ruminants, that use of multisite sampling can detect interorgan changes that would be undetectable using single site sampling. Fed and one-day fasted animals had similar arterial concentrations of BOHB; however, alimentary ketogenesis was depressed by 50% in one-day fasted animals but compensated for by a 43% increase in hepatic ketogenesis. This compensation by the liver represents physiological changes that can not be seen in single site samples.

Comparisons of Dietary Constituents

Comparison of dietary constituents has been evaluated using multicatheterization technique, with emphasis in the relation between diets containing differing levels of concentrate. Gross et al. (1988)

studied glucose turnover as it relates to starch disappearance in the small intestine. This study was a comparison of wheat vs. sorghum with an intermediate 50:50 mix. Used in combination with values for small intestinal digestion of starch it was concluded that 2-15% of the starch absorbed by small intestine appeared as glucose in the portal blood. These values, in conjunction with other data (Huntington, 1983; Huntington and Prior, 1983), suggest a small contribution to total glucose supply is made by net portal absorption. Bergman et al. (1970) also reported that portal absorption of glucose only accounts for one-fifth of total body turnover of glucose.

Janes et al. (1985) equipped sheep with a catheter in the cranial mesenteric vein posterior to the joining of the splenic and mesenteric veins to form the portal. Placement of the catheter at this point allowed for sampling of blood draining the small intestine only. Data from this research showed appreciable amounts of glucose being released into cranial mesenteric blood. This information along with previously cited material suggests extensive use of glucose by rumen epithelial tissue resulting in total portal glucose values accounting for little of the total body glucose supply.

Another area of research in which use of multicatheterization has been used extensively is in evaluation of diets and physiological conditions affecting amino acid and other nitrogen containing compounds. Huntington (1989) studied differences in flux of nitrogenous compounds in cattle fed alfalfa hay or a high concentrate low nitrogen diet. Data suggested a diet consisting of high concentrate and low nitrogen depressed hepatic urea synthesis, increased percent hepatic urea

synthesis transferred to the gut and changed the site of ruminal urea transfer from saliva to the rumen wall. Gross et al. (1988) reported that feeds degraded rapidly and extensively in the rumen also tend to have greater nitrogen digestibility, as indicated by trends in increased urea recycling and additional alpha-amino nitrogen flux. These data agree with Huntington and Reynolds (1986) who found greater transfer of urea from the bloodstream to the rumen in steers fed a high grain diet when compared to those receiving alfalfa.

Conclusion

Use of the multicatheterization technique will allow for identifying quantitatively amounts of energy released by the gut on diets of differing levels of structural carbohydrates. Furthermore, nitrogen released by the portal drained viscera and subsequently taken up for conversion to urea by the liver can be calculated. These calculations allow conclusions to be made as to whether levels of structural carbohydrates and acid detergent insoluble nitrogen (ADIN) adversely affect the amounts of energy and protein that are available from feedstuffs.

Evaluation of DDGS by the use of multicatheterization will allow for the direct calclation of amounts of energy released from the gut in the form of volatile fatty acids, free fatty acids and ketone bodies in this way allowing for inferences to be made about availability of energy in feeds containing DDGS vs. SBM. Literature seems to indiate that fiber contained in DDGS is available for utilization, based on comparisons with corn bran. Samples from multicatheterized sheep will provide information

on liver activity and hindquarter net fluxes of energy metabolites, i.e. conversion and de novo production of ketone bodies, as well as levels of free fatty acid release by hindquarter muscle and adipose. Speculation has been made as to availability of nitrogen bound as ADIN. Evaluation of alpha-amino nitrogen released by portal drained viscera and subsequent conversion to urea by the liver, will give some insight into the availability of ADIN.

In vivo isolation of tissues is possible with multicatheterization, although the tissues are still under direct influence by hormones.

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PART I

INTERORGAN FLUXES OF NITROGENOUS COMPOUNDS IN RUMINANTS FED CORN DISTILLERS DRIED GRAINS WITH SOLUBLES, OR SOYBEAN MEAL FED SEPARATELY OR IN COMBINATION

Abstract

To determine the effects of increasing levels of ADIN on an interorgan net flux of nitrogenous compounds diets containing soybean meal (SBM), corn distillers dried grains with solubles (DDGS) or a 50:50 combination of SBM and DDGS as protein sources. Fifteen experiments were done on 9 mature, nonpregnant, nonlactating crossbred ewes, equipped with chronic indwelling catheters in the caudal aorta and caudal vena cava, portal, hepatic and mesenteric veins. Ewes were fed once daily and consumed a minimum of 1.1 and a maximum of 2.0 kg per day for a minimum of 9 days prior to experiment days. Paraaminohippurate (PAH) was infused into a mesteric vein, allowed to equilibrate, and six samples were taken simultaneously at 30 min intervals from the femoral artery, portal, hepatic and femoral veins. Samples were analyzed immediately for PAH (for blood flow), plasma was harvested and frozen for later analysis of ammonia nitrogen (AMM), urea nitrogen (UN) and alpha-amino nitrogen (AAN). Fluxes of each metabolite were obtained by the multiplication of venoarterial differences and blood flow rates. Portal drained viscera (PDV) uptake of UN on SBM was 12.30 umol/h and increased to 15.34 and 23.23 umol/h when fed SBM + DDGS and DDGS ($P<.1$). Hepatic (HEP) UN release was unaffected

by diet (21.09 to 27.73 umol/h). Gut release of AN was 13.80 umol/h on SBM and decrease to 11.65 and 8.70 umol/h on on SBM + DDGS and DDGS ($P<.05$). Hepatic uptake of AN was 16.29 umol/h on SBM and decreased to 14.66 and 10.59 umol/h on SBM + DDGS and DDGS ($P<.01$). Hepatic UN release from non-ammonia sources was 21.90, 17.40 and 22.46 um/h for SBM, SBM + DDGS and DDGS ($P>.6$). Cautious interpretation of the data might be that ruminally undegradable protein is available for small intestinal absorption and susequent hepatic metabolism.

Introduction

Corn distillers dried grains with solubles (DDGS) is a byproduct of the ethanol production industry. During the fermentation of corn, much of the soluble carbohydrate (starch) is removed, converting a once high energy feed into a moderately high protein supplement. Firkins et al. (1986) reported that heating DDGS during the distillation process increased ADIN of DDGS over the orginal corn grain. Similar heat damages in forages has been identified as a contributing factor to increased amounts of acid detergent insoluble nitrogen (ADIN) in heated forages as compared to their non-heated counterparts (Thomas 1976a, 1976b; Yu and Veira, 1977; Yu et al., 1977). Since soybean meal (SBM) sets the standard for protein sources used in animal feeding it is essential to compare DDGS to SBM when evaluating DDGS as a protein source.

Ruminally undegradable intake protein (UIP) contained in distillers grains and its ability to escape rumen degradation has been well documented (Klopfenstein et al. 1976; Satter et al. 1977; Waller et al.

1980; Klopfenstein et al. 1981). However, literature is scarce concerning availability and subsequent absorption of nitrogen from protein sources high in ADIN. Therefore, this research was conducted to determine N absorption by the gut, and subsequent utilization by liver and peripheral tissue in diets supplemented with DDGS.

Materials and Methods

Fifteen experiments were conducted on a total of nine mature, nonpregnant, nonlactating crossbred ewes, weighing approximately 50 kg. Ewes were kept in pens individual 1.8 x 3.0 m pens with temperature ranging between 19-25 C and were subjected to natural lighting. Ewes were randomly assigned to one of three isocaloric, isonitrogenous diets. Diets were corn cob based with protein supplied by soybean meal (SBM), corn distillers dried distillers grains with solubles (DDGS), or a combination of 50% DDGS and 50% SBM (DDGS + SBM), mixed on a protein basis (Table 1, Appendix). Diets were balanced for calcium, and phosphorus. Ewes were fed once daily and consumed a minimum of 1.1 kg/d for at least ten days before each blood collection period.

Ewes were equipped with chronic indwelling catheters in the portal, hepatic, caudal vena caval, and mesenteric veins and the caudal aortic artery at least 2 weeks prior to starting each experiments. Surgical procedure was performed as described by Katz and Bergman (1969) with modifications reported by Zanzalari et al. (1990). Experiments were initiated at 0900 h with infusion of para-aminohippurate (PAH, 1.5% at 0.764 ml/min) into a mesenteric vein. Following a 1-h equilibration time,

a series of six 15 ml blood samples were taken simultaneously from the caudal aorta, hepatic, portal and caudal vena caval veins at 30-min intervals. Catheter patency was maintained with 6% Na_2EDTA solution between sampling periods and experiments. All samples were immersed immediately in ice to halt any reaction and were treated in accordance with assay procedures.

Samples underwent same day analyses of whole blood packed cell volume, PAH. Plasma was subsequently frozen for later determination of ammonia-nitrogen (AN), blood urea-nitrogen (BUN) and alpha-amino N. Infusion of PAH into a mesenteric vein permits the measurement of blood flow across splanchnic tissues but not across peripheral hindquarters. Therefore, immediately following the final sample, PAH was infused into the caudal aorta and, after a 30-min equilibration period, three additional samples were taken at 10-min intervals from the jugular and caudal vena caval veins. Since little PAH is metabolized by cerebral tissues, jugular PAH concentrations approximate those of arterial blood.

PAH was determined as described by Kaufman and Bergman (1971). Whole blood flow rates were calculated by dividing the infusion rate of PAH (milligrams per minute) by the venoarterial concentration differences (milligrams per liter) across each specific tissue. Hepatic arterial flow was calculated by subtracting portal blood flow from that of the hepatic vein. Plasma flows were obrained by subtracting that portion of the flow represented by the packed cell volume.

Plasma AN was assayed for incorporating a procedure described by McCullough (1967). Determination of BUN was accomplished by the use of Sigma Urea Nitrogen kit (Sigma Chemical Co., St. Louis, MO). Plasma

alpha-amino N concentrations were analyzed using the following adaptation of spectrophometric methods for the determination of alpha-amino nitrogen (Satake et al. 1960 and Palmer and Peters 1969). Stored plasma was thawed in a standard refrigerator, 500ul of the plasma sample was deproteinized using equal volume of 1M $HClO_4$ and diluted with 1ml double distilled H_2O. Samples were then spun for 15 min in at 13000 x G. Samples were buffered by the addition of 100ul 1M NaOH and 500ul sodium borate buffer to 250ul of supernatent. All subsequent steps conducted in the absence of light. An addition of 500ul 2,4,6-trinitrobenzene 1-sulfonic acid was made to each sample. Samples were incubated at 37° for exactly 19 min. Reaction was then halted by the addition of 500ul 1N HCl and following read at 340nm.

Methods used to calculate net fluxes of AN, BUN and alpha-amino N portal drained visceral, hepatic, total splanchnic, and lower hindquarter tissues were described by Heitmann and Fernandez (1986). The plasma or whole blood venoarterial concentration differences for each metabolite (units of concentration per liter) for a specific tissue were multiplied by that tissue's plasma or whole blood flow rate (liters per minute). A positive flux indicates net release, while a negative flux is indicative of a net uptake of the metabolite by a specific tissue.

The extraction ratio is the fraction of the metabolite presented to the tissue (tissue blood flow multiplied by concentration in the arterial input) that is taken up by the tissue (net flux). Extraction ratios for each metabolite and each tissue were calculated. Significant changes in extraction ratios between treatments indicate changes in net flux are at least due in part to some physiological mechanism resulting from an

imposed treatment and are not solely based on changes in metabolite concentration or blood flow.

Data analysis was accomplished by analysis of variance, using the model which included (animal, treatment, and animal x treatment interaction) as sources of variation. In all cases the animal was considered as the experimental unit. Venoarterial differences were calculated and differences determined by Student - Newman - Kuels multiple range test (Snedecor and Cochran, 1967).

Results

Samples of each diet were analyzed for crude protein, NDF, ADF, ADL and gross energy (Table 2, Appendix). Diets were found to be isocaloric and isonitrogenous. DDGS was higher in NDF, ADF and ADL with SBM being the lowest and DDGS + SBM falling as an intermediate in each case.

Whole blood flow rates across splanchnic and peripheral tissues are shown in Table 3, Appendix. There were no significant effects of diet on blood flow rates. Portal vein blood flow ranged from 1.54 to 1.81 l/min, which represent approximately 80% of hepatic vein flow of 1.91 to 2.25 l/min. Hepatic artery data has been omitted for simplification but can be easily calculated by subtracting portal flow from hepatic flow. Blood flow rates across the hindquarters ranged from 1.39 to 1.89 l/min. Blood flow rates are similar to those reported for sheep by Heitmann et al. (1986) as well as Heitmann and Bergman (1980) and when adjusted for body weight, fell within the range reported in cows as mesured by indicator

dilution (Huntington, 1990) or electromagnetic flowmetry (Durand et al., 1988).

Table 4 (Appendix) contains AN concentrations and net fluxes. Release of AN by the gut was less (P<.05) on DDGS (8.70 umol/h) than SBM (13.8 umol/h), with DDGS + SBM uptake. Liver extraction of AN was elevated (P<.1) for animals fed SBM in comparison to animals fed DDGS. Liver uptake of AN was 16.29 umol/h, vs. 10.59 umol/h and 14.66 umol/h for SBM, DDGS and DDGS + SBM respectively. Total splanchnic uptake of AN was more than 50% higher on DDGS + SBM than on SBM alone (P<.10), DDGS was uptake was at an intermediate level. Ewes receiving DDGS had higher arterial (P<.10) AN concentrations than those receiving DDGS + SBM. Hindquater fluxes of AN were not significantly different than zero.

Gut uptake of BUN on DDGS diets was 89% higher (P<.10) than of uptake on SBM diets (Table 5, Appendix). Liver and TSP release of BUN was similar across treatments, however, arterial concentrations of BUN were lower (P<.01) on DDGS + SBM than on DDGS or SBM (2.88 uM vs. 4.50 uM, 4.91 uM). Hepatic BUN release that was from non-ammonia sources ranged from 17.40 umol/h on DDGS + SBM, to 22.46 umol/h on DDGS but were similar across all three treatments.

Gut release of alpha-amino N (Table 6, Appendix) was similar for all diets (SBM 8.40um/h, DDGS 8.40um/h and DDGS + SBM 13.20um/h). Uptake of alpha-amino N by the liver was 86% greater when ewes were fed DDGS + SBM than when fed SBM (P<.1). Hepatic release eliminated a large enough amount of alpha-amino N resulting TSP fluxes that were not significantly different from zero. RUMP alpha-amino N flux was zero, thus arterial concentrations were not significantly different.

Discussion

Similarity of blood flow rates across all tissues, regardless of treatment, confirms that all flux differences reported were a result of veno-arterial differences and were not a function of blood or plasma flow rates.

Increased AN release by the gut when ewes were fed SBM compared to DDGS was consistent with expectations. As reported by Santos et al. (1983) in lactating dairy cows, DDGS based diet resulted in 54% rumen protein escape compared to 30% escape when SBM was fed; therefore, depressed release of ammonia by PDV would be expected due to high UIP reported by these researchers. Feeding of diets supplemented with DDGS + SBM resulted in intermediate release of AN by the PDV. Although rumen ammonia values were not measured it can be expected that greater PDV release of ammonia can be translated to greater rumen fermentation. Liver uptake of AN was, in all cases, greater than PDV release indicating HEP removal of all dietary sources of AN and removal of endogenous recirculated AN.

Kennedy and Milligan (1980) noted urea uptake by the reticulorumen and hindgut was a significant source of nitrogen for microbial growth, and that transfer of urea to the rumen was positively correlated with ruminal digestible organic matter. Uptake of UN into portal blood was by 88% than those fed SBM and 51% greater than those receiving DDGS + SBM. These uptake patterns could be attributed to rumen concentrations of ammonia (Kennedy and Milligan, 1980), PDV uptake of urea was inversely related to rumen ammonia concentrations (Kennedy and Milligan, 1980). Increased

transfer of UN into the gut may be due to an increase in numbers and activity of ureolytic bacteria and decreased rumen ammonia concentrations (Cheng and Wallace, 1979; Egan, 1980; Kennedy et al., 1981; Javorsky et al., 1987). Arterial UN concentrations were lowered on DDGS + SBM despite similar TSP release, however, no physiological explanation could be hypothesized from data collected in this study.

Utilization by ruminants of ADIN contained in DDGS was reported by Britton et al. (1986). Questions were raised by these researchers as to the validity of ADIN measurements made in evaluation of both in DDGS in diets and in fecal samples collected. Very low quantities of ADIN was present in feces when feeds of high ADIN were fed, and greater than 100% recovery of ADIN occurred in feeds low in ADIN. Because of low recovery Britton and coworkers (1986) suggested that an alternative method of quatitating indigestible nitrogen must be developed. However, data from this study suggested that ADIN contained in DDGS was available for absorption.

Due to similarities in alpha-amino N release by the PDV it would seem that ADIN contained in DDGS was available for absorption.

PART I LIST OF REFERENCES

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PART I APPENDIX

TABLE 1

COMPOSITION OF DIETS[a]

	DDGS	DDGS + SBM[b]	SBM
DDGS	33.85	16.80	-----
SBM	-----	9.75	19.40
Corncobs	50.00	50.00	50.00
Corn Starch	5.00	12.30	19.45
Molasses	10.00	10.00	10.00
Dicalcium Phosphate	.70	.70	.70
Limestone	.20	.20	.20
Trace Mineralezed Salt	.25	.25	.25

[a]% dry matter.

[b]50% of the crude protein was from DDGS and 50% from SBM.

TABLE 2

ANALYSIS OF DIETS

ITEM	DDGS	DDGS + SBM	SBM
DM, %	94.75	94.83	94.07
Proportion of DM, %			
CP	14.92	15.38	15.14
NDF	31.56	50.34	45.46
ADF	15.10	22.42	22.79
ADL	1.99	4.34	3.82
Gross Energy, Kcal/g DM	4.16	4.53	4.38

TABLE 3

BLOOD FLOW RATES (liters/min)

	Portal Vein	Hepatic Vein	Femoral Vein
DDGS	1.54	1.91	1.55
DDGS + SBM	1.81	2.22	1.89
SBM	1.80	2.25	1.39

TABLE 4

AMMONIA ARTERIAL CONCENTRATIONS AND NET FLUXES

Treatment	Artery	Portal-drained Viscera	Liver	Total Splanchnic	Lower Hindquarters
	umol	----------	----------	umol/h ----------	----------
SBM	.18[a,b]	13.80	-16.29[c]	-1.46[a]	-.34
DDGS	.23[a]	8.70	-10.59[d]	-1.89[a,b]	.13
DDGS + SBM	.15[b]	11.65	-14.66[c,d]	-3.00[b]	-.13
SEM	.04	1.82	2.14	.56	.39

[a,b]$p<.1$.

[c,d]$p<.05$.

TABLE 5

UREA ARTERIAL CONCENTRATIONS AND NET FLUXES

Treatment	Artery	Portal-drained Viscera	Liver	Urea-N	Total Splanchnic	Lower Hindquarters
	umol	----------	----------	umol/h	----------	----------
SBM	4.91[c]	-12.30[a]	25.89	21.90	13.59	-10.67
DDGS	4.50[c]	-23.23[b]	27.73	22.46	4.50	-10.24
DDGS + SBM	2.88[d]	-15.34[a,b]	21.09	17.40	5.70	-4.16
SEM	.37	5.32	6.00	8.01	5.99	6.72

[a,b]$p<.1$.

[c,d]$p<.01$.

TABLE 6

ALPHA AMINO-N ARTERIAL CONCENTRATIONS AND NET FLUXES

Treatment	Artery	Portal-drained Viscera	Liver	Total Splanchnic	Lower Hindquarters
	umol	----------	umol/h	----------	
SBM	2.74	8.40	-8.40[a]	.00	.00
DDGS	2.62	8.40	-12.60[a,b]	-4.20	-1.20
DDGS + SBM	2.60	13.20	-15.60[b]	-3.00	-1.20
SEM	.64	2.66	2.54	3.84	2.15

[a,b]$p<.1$.

PART II

PORTAL DRAINED VISCERA (PDV), HEPATIC (HEP) AND HINDQUARTER (RUMP) NET FLUX OF ENERGY METABOLITES WITH FEEDING OF DISTILLERS GRAINS AND/OR SOYBEAN MEAL

Abstract

To determine effects of feeding increasing concentrations of NDF and ADF on interorgan fluxes of energy metabolites, 15 experiments were done on 9 mature, nonpregnant, nonlactating crossbred ewes. Ewes were equipped with chronic indwelling catheters in the caudal aorta and caudal vena cava, portal, hepatic and mesenteric veins. Ewes received one of three isocaloric diets (1.1 kg/d) containing soybean meal (SBM), corn distillers dried grains with solubles (DDGS) or a 50:50 mix of SBM and DDGS, once daily for a minimum of 9 days prior to blood collection. Paraaminohippurate (PAH), was infused into a mesenteric vein and allowed to equilibrate for 1 h. Subsequently, samples were taken simultaneously from the caudal aorta and caudal vena cava, portal and hepatic veins at 30 min intervals for 2.5 h. Samples were analyzed for PAH, glucose (GLU), free fatty acids (FFA), acetoacetate (AC) and beta-hydroxybutyrate (BOHB). Plasma was harvested prior to VFA analyses. Arterial GLU concentrations decreased ($P<.01$) when ewes received DDGS, from 53 mg/100ml on both SBM and DDGS + SBM to 48 mg/100ml. This was due to a slight increase in RUMP utilization. An almost two fold increase ($P<.01$) was noted in FFA arterial concentrations on DDGS + SBM (257 uM on SBM, 260 uM on DDGS vs.

421 uM on DDGS + SBM). Hindquarter release of FFA increased (P<.01) from 10.5 umol/min when ewes received SBM and 15.5 umol/min on DDGS to 57.9 umol/min when ewes received DDGS + SBM. Additionally, a two fold increase (P<.01) in hepatic uptake of FFA resulted when ewes received DDGS + SBM, however, percent of FFA removed by the liver remained constant in each treatment. No physiologically significant changes were observed in either AC or BOHB concentrations or net flux rates. Addition of DDGS decreased (P<.01) acetate (AT) and butyrate (BU) arterial concentrations (AT = 929, 725 and 620 uM; BU = 11, 7 and 5 uM on SBM, DDGS and DDGS + SBM, respectively) due to decreased PDV release (AT = 1267, 1010 and 1065 umol/min; BU = 46, 34 and 19 umol/min on SBM, DDGS and DDGS + SBM, respectively). Liver extraction of AT and BU was 14 and 72% that of the PDV release resulting in significant total splanchnic production and subsequent decreased (P<.05) RUMP utilization (AT = 502, 133 and 145 umol/min; BU = 5, 2 and 1 umol/min on SBM, DDGS and DDGS + SBM). There were no changes in propionate or isobutyrate concentrations or significant RUMP utilization since HEP uptake of these VFA was 90-100% of PDV release. AT and BU changes may have been due to decreased rumen fermentation with increased concentrations of ADF and NDF.

Introduction

Corn distillers dried grains with solubles (DDGS) have been researched for over forty years as a byproduct feed source. However, the vast majority of research in ruminants has focused on DDGS as a source of undegradable intake protein (UIP), with few studies addressing the energy

availability of DDGS. Klopfenstein (1985) suggested DDGS may supply similar and in some cases greater amounts of energy than corn grain.

Since DDGS is classified as a protein supplement, and soybean meal (SBM) sets the standard for protein sources used in animal feeding it is essential to compare DDGS to SBM when evaluating DDGS as a protein source.

Addition of DDGS to diets, may increase amounts of carbohydrate, particularly ADF and NDF escaping ruminal degradation. Much research has shown that extensive degradation of carbohydrate in DDGS can occur in the cecum and proximal colon, as well as an increased efficiency in utilization of starch (Weber, 1983). Dixon and Nolan (1982) and Hoover (1978) reported significant nutrient digestion and absorption can occur post-ruminally.

The following study was designed to evaluate energy availability of DDGS, through the evaluation of fluxes in energy metabolites among sheep fed corn cob based diets incorporating SBM, DDGS and a combination of these two as protein sources.

Materials and Methods

Fifteen experiments were conducted on nine mature, nonpregnant, nonlactating crossbred ewes, weighing approximately 50 kg. Ewes were kept in individual 1.8 x 3.0 m pens with temperature ranging between 19-25 C and subjected to natural lighting. Ewes were assigned randomly to one of three isocaloric, isonitrogenous diets, based on corn cobs supplemented with SBM, DDGS, or a combination of 50% DDGS and 50% SBM (DDGS + SBM), mixed on a protein basis (Table 1, Appendix). Diets were balanced for

calcium, and phosphorus. Representative diet samples were analyzed for ADF, NDF, ADL, crude protein and gross energy (Table 2, Appenndix). Diets were isocaloric and isonitrogenous, and DDGS was higher in ADF, NDF and ADL, with SBM being the lowest and DDGS + SBM being an intermediate in each case. Ewes were fed once daily and consumed a minimum of 1.1 and a maximum of 2.0 kg/d for at least 10 d prior to blood collection.

Ewes were equipped with chronic indwelling catheters in the portal, hepatic, caudal vena caval, and mesenteric veins and the caudal aortic artery at least 2 weeks prior to each experiment. Surgical procedure was performed as described by Katz and Bergman (1969) and modified by Zanzalari et al. (1990). Experiments were initiated at 0900 h with infusion of para-aminohippurate (PAH, 0.5% at 0.764 ml/min) into a mesenteric vein. Following a 1-h equilibration period, a series of six 15 ml blood samples were taken simultaneously from the caudal aorta, hepatic, portal and caudal vena caval veins at 30-min intervals. Catheter patency was maintained with 6% Na_2EDTA solution between sampling periods and experiments. Use of heparin was avoided to prevent lipoprotein lipase activation. All samples were immersed immediately in ice and were treated in accordance with assay procedures described below.

Samples underwent same day analyses of whole blood packed cell volume, PAH, glucose, Beta-hydroxybutyrate, and acetoacetate as well as plasma free fatty acids (FFA). Plasma was subsequently frozen for later determination of volatile fatty acids (VFA). Infusion of PAH into a mesenteric vein permits the measurement of blood flow across splanchnic tissues but not across peripheral hindquarters. Therefore, immediately following the final sample, PAH was infused into the caudal aorta and,

after a 30-min equilibration period, three additional samples were taken at 10-min intervals from the jugular and caudal vena caval veins. Since little PAH is metabolized by cerebral tissues, jugular PAH concentrations approximate those of arterial blood.

Determination of PAH was done as described by Kaufman and Bergman (1971). Two ml of blood was added to an equal volume of cold 1M $HClO_4$ in preparation for acetoacetate and Beta-hydroxybutyrate analysis. The tubes were vortexed, centrifuged at 4 C and 1500 x g for 25 min and buffered to a final pH of 6.4 - 7.6 with cold 1M KOH. The samples were analyzed immediately by the enzymatic assay of Williamson and Mellanby (1965). Plasma FFA were analyzed with a WAKO NEFA-C kit (WAKO Chemical USA, Dallas, TX) and blood glucose was determined with a Sigma glucostat 510 kit (Sigma Chemical Co., St. Louis, MO). Plasma volatile fatty acids were assayed by gas chromatography utilizing a procedure described by Quigley and Heitmann (1990).

Whole blood flow rates were calculated by dividing the infusion rate of PAH (mg/min) by venoarterial concentration differences (mg/L) across each tissue. Hepatic arterial flow was calculated by subtracting portal blood flow from that of the hepatic vein. Plasma flows were obtained by subtracting the portion of the flow represented by the packed cell volume.

Methods of calculation for net fluxes of glucose, FFA, acetoacetate, B-hydroxybutyrate, and VFA across portal drained visceral, hepatic, total splanchnic, and lower hindquarter tissues has been described by Heitmann and Fernandez (1986). Plasma or whole blood venoarterial concentration differences for each metabolite (units of concentration per liter) for a specific tissue are multiplied by that tissue's plasma or whole blood flow

rate (liters per minute). It is important to note that a positive flux indicates net release, while a negative flux is indicative of a net uptake of the metabolite by a specific tissue. Extraction ratios for each metabolite and each tissue were calculated. The extraction ratio is the fraction of the metabolite presented to the tissue (tissue blood flow multiplied by concentration in the arterial input) that is taken up by the tissue (net flux). Significant changes in extraction ratios between treatments indicate changes in net flux with treatment are at least due in part to some physiological mechanism and are not solely based on changes in metabolite concentarion or blood flow.

Data analysis was accomplished by analysis of variance, using the model which included included animal, treatment, and animal x treatment interaction as sources of variation. In all cases the animal was considered as the experimental unit. Venoarterial differences were calculated and differences determined by Student - Newman - Kuels multiple range test (Snedecor and Cochran, 1967).

Results

Whole blood flow rates across splanchnic and peripheral tissues (Table 3, Appendix) were unaffected by treatment. Portal vein blood flow ranged from 1.54-1.81 l/min and was approximately 80% of hepatic vein flow (1.91-2.25 l/min). Hepatic artery data has been omitted for simplification but can be easily calculated by subtracting portal flow from hepatic flow. Blood flow rates across hindquarters ranged from 1.39-1.89 l/min. All blood flow rates reported here are similar to those

reported for sheep by Heitmann et al. (1986) and Heitmann and Bergman (1980) and, when adjusted for body weight, fell within the range reported in cows as measured by indicator dilution (Huntington, 1990) or electromagnetic flowmetry (Durand et al. 1988).

Whole blood glucose concentrations in the artery decreased significantly ($P<.01$), by 11%, in animals receiving the diet containing DDGS (Table 4, Appendix). This is, in part, due to an increase in portal drained viscera (PDV) uptake. Arterial glucose concentrations were similar in those animals receiving DDGS + SBM and SBM. This was despite a decrease in PDV uptake of glucose on the DDGS + SBM diet, however, this decrease is offset by an almost two fold increase in hindquarter (RUMP) utilization -21.5 vs. -11.1 ($P<.1$) and also compensated for by a 75% (31.8 vs. 18.2 mmol/h) increase in total splanchnic (TSP) release ($P<.1$).

Arterial FFA concentrations increased approximately 63% ($P<.01$) in animals fed DDGS + SBM (Table 5, Appendix), which can be attributed to a 4 - 5 fold increase in RUMP release of FFA (Table 5). This increase was seen although a significant increase in liver uptake ($P<.01$) occurred. Hindquarter measurements represent only about one-third of total body muscle and adipose, therefore, a five fold increase in hindquarter release may have indicated a significant increase in total FFA body release, which in turn, increased arterial concentrations. Liver uptake of FFA was passive and concentration dependent since hepatic extraction ratios remained constant and ranged from 13 - 15% of that presented to the liver.

Both acetoacetate and Beta-hydroxybutyrate arterial concentrations were similar across diets (Tables 6 and 7, Appendix). There were no

differences observed in acetoacetate flux across tissue beds. Uptake of B-hydroxybutyrate declined (P<.05) in animals fed DDGS.

Arterial concentrations of acetate decreased significantly (P<.01) from .929 mM on sheep fed SBM to .725 and .620 mM in sheep fed DDGS and DDGS + SBM, respectively. Decreases occurred despite a significant decrease in RUMP uptake of acetate .458 mM for SBM vs. .133 and .145 mM for DDGS and DDGS + SBM (P<.01), respectively.

Arterial concentrations of propionate were similar across diets (Table 8, Appendix). An increase (P<.01) in PDV release was observed when ewes were fed DDGS (Table 8). Hepatic uptake of propionate was higher (P<.01) in sheep fed DDGS than those fed SBM (P<.05), and DDGS + SBM (P<.01). Hepatic uptake ranged from 0.29 mmol/min on DDGS, .19 mmol/min on SBM, and .16 mmol/min on DDGS + SBM. In every case the liver removed a minimum of 90% of the propionate released by the gut. Resulting TSP releases of propionate were not significantly different than zero. Furthermore, no reportable amounts of propionate were used by peripheral tissues.

Concentrations of butyrate in the artery decreased significantly (P<.05) from .011 mM to .007 mM in animals receiving SBM vs. DDGS. In addition, concentrations decreased 83% (.011 mM vs. .005 mM) when comparing animals fed SBM to DDGS + SBM (P<.01). Increasing arterial butyrate concentrations in sheep fed SBM are primarily the result of a increase in PDV release of butyrate. Ewes fed SBM had a significantly greater gut release of butyrate, .046 mmol/min, than DDGS + SBM, 0.019 mmol/min (P<.01), while the DDGS release was a nonsignificant intermediate, .034 mmol/min. Liver uptake of butyrate increased in a

similar stepwise manner with uptake values of .013, .024 and .036 for DDGS + SBM, DDGS, and SBM. It is important to recognize HEP extraction ratios were similar for all treatments, approximately 40%.

Isobutyrate concentrations in the artery were similar for all three treatments ranging from .002 mM to .003 mM. Gut release of isobutyrate was greater P<.01) in SBM, .010 mmol/min, than DDGS + SBM, .003 mmol/min. However, on DDGS a HEP uptake of isobutyrate of .001 mmol/min occurred, this was significantly different (P<.001) than SBM gut release. In sheep fed SBM an uptake of .010 mmol/min was significantly greater than on DDGS + SBM, at .003 mmol/min (P<.001). Hepatic uptake was similar to PDV release resulting in a release of isobutyrate of .001 mmol/min, thereby, leaving TSP values of zero mmol/min for all treatments.

In ewes fed SBM arterial concentrations of valerate were greater (P<.05) than those fed DDGS, (.003 mM vs .000 mM) while DDGS + SBM was. No significant differences were seen in PDV release of HEP net flux. Portal drained viscera release ranged from .002 mmol/min to .006 mmol/min. In both SBM and DDGS + SBM, HEP release of valerate occurred while in sheep fed DDGS an uptake was indicated. This HEP uptake resulted in significantly lower TSP release of valerate (.002 mmol/min for DDGS and .008 mmol/min for SBM) (P<.05), again with the DDGS + SBM an intermediate (.004 mmol/min). Isovalerate concentrations in the artery increased (P<.01) to 0.002 mM in ewes fed SBM, from .000 mM in those receiving DDGS. PDV release of isovalerate were higher (P<.05) on SBM, .036 mmol/min than either DDGS, .020 mmol/min or DDGS + SBM, .024 mmol/min. No significant differences occurred in HEP uptake of isovalerate or HEP extraction ratios. However, calculation of HEP extraction ratios revealed a stepwise

increase with DDGS + SBM being the lowest and DDGS as the highest, with SBM as an intermediate. This increase still allowed for nonsignificant TSP differences in TSP release of isovalerate ranging from .003 mmol/min for DDGS, .005 mmol/min on DDGS + SBM, and .007 mmol/min for SBM.

Discussion

This research, to our knowledge, is the first to address interorgan flux of traditional energy metabolites with the feeding of increasing structural carbohydrates, ie. replacement of SBM by DDGS. Total energy released by the gut into portal blood in Kcal/d was similar for animals fed SBM or DDGS (Table 9, Appendix). However, a decrease in energy available for utilization from the portal drained viscera was seen when ewes received DDGS + SBM. Percentage of total Kcal/d release of energy could be accounted for by VFA was highest on SBM, lowest on DDGS + SBM, and intermediate when ewes were fed DDGS. Therefore, increasing ADF and NDF content of the diet concomitantly decreased proportion of available energy supplied by VFA.

Increased arterial concentrations of acetate when ewes received SBM would indicate a more extensive fermentative digestion in the rumen. Increased arterial concentrations may have been due to increased PDV release of acetate when sheep were fed SBM, although elevated peripheral usage of acetate was seen it was not extensive enough to lower arterial concentrations to the level of the other two treatments. Hindquarter extraction ratios were higher ($P<.05$) in ewes fed SBM, 26.2% of that acetate presented to the hindquarter was removed, as compared with 14.7%

for DDGS and 13.8% for DDGS + SBM. Following in a similar pattern was butyrate, with increased PDV release of butyrate with the feeding of SBM and, significant peripheral usage of butyrate, when ewes received SBM or DDGS. Increased PDV release of propionate on DDGS + SBM suggests a negative associative effect when DDGS and SBM are fed at a 1:1 ratio, on a protein basis. A decrease in rumen pH by feeding DDGS would support the increased percentage of Entodinia inhabiting the rumen (Hungate, 1966), thereby increasing rumen propionate, followed by a concomittant increase in release of propionate to the portal blood.

Ketone body PDV release in Kcal/d (Table 9) is consistent with Baird et al. (1975) who reported approximately 170 kcal/d released by the gut could be accounted for by ketones. Further, circulating concentrations of beta-hydroxybutyrate are similar to those reported by others (Heitmann et al. 1986; Roe et al. 1966; Katz and Bergman 1969; Brockman 1976; Kaufman and Bergman 1971; Jarrett et al. 1976).

Decreased arterial glucose concentrations in ewes fed DDGS appeared to be a function of incresed RUMP uptake of glucose, although this trend was not significant. Because RUMP measurements represent approximately one third total body mass, increased RUMP uptake could indicate to a significant total body uptake of glucose, resulting in a decreased in circulating concentrations of glucose.

A two fold increase in arterial FFA concentrations coupled with a four to five fold increase in RUMP release of FFA when animals were fed DDGS + SBM indicated lipolysis, and may support the hypothesis that a negative associative effect occurred when DDGS and SBM were fed simultaneously. Arterial concentrations were increased as a result of the

consistent removal of approximately 15% of FFA by the liver. Detection of lipolysis is accomplished with catheter position in the distal caudal vena cava, allowing for the sampling of the external iliac vein, deep circumflex, internal iliac veins and the femoral veins. Additionally, the external iliac drains significant interfascicular, intermuscular and subcutaneous adipose tissue (Heitmann et al. 1987).

Based on data reported in this study DDGS seems to be equal to SBM on an energy basis. Metabolite differences exist, but energy supplied by each diet are similar. When DDGS and SBM are fed in combination, energy released from the PDV declines, suggesting an unknown relationship between the feedstuffs, resulting in a lowering of energy metabolites being released by the PDV.

PART II LIST OF REFERENCES

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PART II APPENDIX

TABLE 1

COMPOSITION OF DIETS[a]

	SBM	DDGS	DDGS + SBM[b]
DDGS	-----	33.85	16.80
SBM	19.40	-----	9.75
Corncobs	50.00	50.00	50.00
Corn Starch	19.45	5.00	12.30
Molasses	10.00	10.00	10.00
Dicalcium Phosphate	.70	.70	.70
Limestone	.20	.20	.20
Trace Mineralized Salt	.25	.25	.25

[a]Percent of DM.

[b]50% of CP from DDGS and 50% from SBM

TABLE 2

ANALYSIS OF DIETS

ITEM	SBM	DDGS	DDGS + SBM
DM, %	94.75	94.83	94.07
Proportion of DM, %			
Crude Protein	14.92	15.38	15.14
NDF	31.56	50.34	45.46
ADF	15.10	22.42	22.79
ADL	1.99	4.34	3.82
Gross Energy, Kcal/g	4.16	4.53	4.38

TABLE 3

BLOOD FLOW RATES

	PORTAL VEIN	HEPATIC VEIN	FEMORAL VEIN	P/H[a]
	----------liters/min----------			
SBM	1.687	2.119	1.470	.796
DDGS	1.601	1.962	1.572	.816
DDGS + SBM	1.913	2.304	1.572	.830

[a]Ratio of portal vein to hepatic vein blood flow.

TABLE 4

GLUCOSE ARTERIAL CONCENTRATIONS AND NET FLUXES

Treatment	Artery	Portal-drained Viscera	Liver	Total Splanchnic	Lower Hindquarters
	mg/100ml	----------mmol/h----------			
SBM	53[d]	-9.0	30.9	21.9[c]	-13.4
DDGS	48[c]	-8.7	34.5	25.9[a]	-20.5
DDGS + SBM	53[d]	-3.4	42.2	38.8[b,d]	-12.8
St. error	1.2	4.0	5.3	4.5	4.2

[a,b]$p<.05$.

[c,d]$p<.01$.

TABLE 5

FREE FATTY ACID ARTIERIAL CONCENTRATIONS AND NET FLUXES

Treatment	Artery	Portal-drained Viscera	Liver	Liver Ext.	Total Splanchnic	Lower Hindquarters
	umol	---umol/min----		%	--------umol/min--------	
SBM	257[b]	15.5	-50.4[b]	13	-34.9[b]	10.5[b]
DDGS	260[b]	18.8	-48.9[b]	14	-30.1[b]	15.5b
DDGS + SBM	421[a]	22.0	-104.4[a]	15	-82.4[a]	57.9[a]
St. error	30.5	5.3	9.9	.01	9.4	9.5

[a,b]p<.0.

TABLE 6

ACETOACETATE ARTERIAL CONCENTRATIONS AND NET FLUXES

Treatment	Artery	Portal-drained Viscera	Liver	Total Splanchnic	Lower Hindquarters
	umol	----------umol/h----------			
SBM	24	27.1	-35.7	-8.7	-8.6
DDGS	20	30.2	-32.4	-2.1	-7.9
DDGS + SBM	20	33.0	-36.9	-3.9	-5.5
St. error	2.1	2.8	2.9	2.9	2.8

TABLE 7

BETA-HYDROXYBUTYRATE ARTERIAL CONCENTRATIONS AND NET FLUXES

Treatment	Artery	Portal-drained Viscera	Liver	Total Splanchnic	Lower Hindquarters
	umol	----------umol/min----------			
SBM	436	164.1	162.9	327.0	-125.8[b]
DDGS	415	210.4	154.6	365.0	- 73.6[a]
DDGS + SBM	401	205.4	187.9	393.3	-140.9[b]
St. error	34.2	23.6	19.9	31.0	17.8

[a,b]$p<.05$

TABLE 8

VFA ARTERIAL CONCENTRATIONS AND NET FLUXES

Artery	Portal-drained Viscera	Liver	Total Splanchic	Lower Hindquarters	
	umol	----------umol/min----------			
ACETATE					
SBM	.929[c]	1.27	-.20	1.02	-.50[c]
DDGS	.725[d]	1.01	-.09	.88	-.13[d]
DDGS + SMB	.620[d]	1.06	-.18	.84	-.15[d]
SEM	.050	.10	.08	.10	
PROPIONATE					
SBM	.03	.20[d]	-.19[a,b]	.01[a,b]	.00
DDGS	.02	.32[c]	-.29[b,c]	.03[b]	.00
DDGS + SBM	.03	.17[d]	-.16[a,b]	.00[a]	.00
SEM	.003	.03	.03	.007	
BUTYRATE					
SBM	.011[a,b]	.046[a]	-.036[c]	.010	-.005
DDGS	.007[b,d]	.034[a,b]	-.024[c,d]	.009	-.002
DDGS + SBM	.005[b,d]	.019[b]	-.013[d]	.006	-.001
SEM	.001	.007	.006	.002	
ISOBUTYRATE					
SBM	.003	.010[c]	-.010[c]	.000	.000
DDGS	.003	-.001[d]	.001[d]	.000	.000
DDGS + SBM	.002	.003[d]	-.003[d]	.000	.000
SEM	.0005	.002	.002	.000	.000
VALERATE					
SBM	.003[a]	.006[a]	.001	.007	-.003
DDGS	.001[b]	.004[a,b]	-.002	.002	.000
DDGS + SBM	.001[b]	.001[b]	.003	.005	.000
SEM	.001	.002	.002	.002	.002
ISOVALERATE					
SBM	.002[a]	.03[a]	-.033[a]	.005[a]	-.002
DDGS	.001[b]	.021[b]	-.020[b]	.002[b]	-.000
DDGS + SBM	.001[b]	.025[b]	-.22[a,b]	.004[a,b]	-.000
SEM	.0003	.004	.004	.001	.000

TABLE 9

ENERGY (kcal/d) RELEASED BY THE PORTAL DRAINED VISCERA

Metabolite	SBM	% of Total	DDGS	% of Total	DDGS + SBM	% of Total
FFA	60.58	8.1	73.47	9.8	85.98	12.4
ACAC	18.77	2.5	20.92	2.8	22.86	3.3
BOHB	114.61	15.3	146.94	19.6	143.45	20.7
Acetate	369.08	49.4	304.55	40.7	317.22	45.9
Propionate	102.05	13.6	154.93	20.7	80.90	11.7
Butyrate	33.97	4.5	24.06	3.2	13.59	2.0
Isobutyrate	7.55	1.0	-.75		2.26	.3
Valerate	5.89	.7	4.91	.7	1.96	.3
Isovalerate	35.33	4.7	19.63	2.6	23.56	3.4
TOTAL	747.83		748.76		691.78	

APPENDIXES

APPENDIX A

PARA-AMINOHIPPURIC ACID ANALYSIS

Reagents for Filtrate

1) Double Distilled (DD) H_2O

2) 20% Trichloroacetic Acid (TCA) (CCl_3COOH)
(Sigma Chemical Co., cat. # T-6399)

200 g CCl_3COOH ---> 1.0 liter DD H_2O

Method for Filtrate

1) Pipette 1.0 ml of blood or standard into 15 ml centrifuge tube containing 5.0 ml DD H_2O.

2) Pipette 5.0 ml of blood or standard mixture into second 15 ml centrifuge tube containing 5.0 ml 20% TCA.

3) Let stand 60 minutes or overnight (can be left at this stage if refrigerated).

4) Filter through Whatman #4 filter paper into 16 X 150 mm glass culture tubes.

5) Add boiling chip to each culture tube and place a marble on top of each tube.

6) Boil over low heat in water bath for 30 minutes after fine bubbles appear in each tube.

7) Cool at room temperature and remove marbles when cool.

8) Standards from 1.5% PAH infusion solution are made along with this using same method as for blood filtrate.

Make up a 1:500 dilution for sheep from 1.5% infusion solution; 1:100 for dog from 0.25% infusion solution (considered 10:10).

From this dilution make up a standard dilution: 20% = 2:10; 40% = 4:10; 60% = 6:10; 80% = 8:10; and 100% = 10:10 (stock dilution). Be sure to run this through the filtrate procedure (do not have to boil).

Blank mixed with half DD H_2O and half TCA (20%).

Reagents for Analysis

1) 1.2 N Hydrocloric Acid (HCL)
(Fisher Scientific Co., cat. # A-144-S)

(1:10 dilution of 12.4 N HCL close enough).

2) Sodium Nitrite ($NaNO_2$)
(Sigma Chmical Co., cat. # S-2252)

100 mg $NaNO_2$ ---> 100 ml H_2

(Freshly made up on day of analysis)

3) Ammonium Sulfamate ($NH_4OSO_2NH_2$)
(Sigma Chemical Co., cat. # A-8670)

500 mg $NH_4OSO_2NH_2$ ---> 100 ml H_2O

(Made up within a month)

4) Coupling Reagent N(-1-Naphthyl)Ethylenediamine
(Sigma Chemica Co., cat. # N-5889)

100 mg $C_{10}H_7NHCH_2CH_2NH_2$ * 7 H_2O ---> 100 ml DD H_2O

(Store in a brown bottle in refrigerator indefinitely)

Analysis

1) Pipette 1.0 ml of filtrate into test tube (in duplicate).

Pipette 1.0 ml of standard into test tube (in duplicate).

Pipette 1.0 ml of blank into test tube (in duplicate).

2) Add 0.2 ml of 1.2 N HCL to all tubes and vortex.

Start time clock and add to all tubes:

0.1 ml sodium nitrite and vortex.

0.1 ml ammonium sulfamate within 3 to 5 minutes and vortex.

0.1 ml coupling reagent within 3 to 5 minutes and vortex.

Wait 10min for color development.

Read absorbance on spectrophotometer at 540 nm.

PAH Stock Solution

1) Para-Aminohippuric Acid (PAH)
(Eastman Kodak corp., cat. # 5704)

2) Sodium Hydroxide (NaOH)
(Fisher Scientific Co., cat. # S-318)

Dissolve each reagent separately in Physiological Sterile Saline (PSS):

44.8 g PAH

10.5 g NaOH

Then add NaOH solution to PAH solution and stir. May have to heat gently to fully dissolve. Filter through Whatman #4 filter paper. Titrate filtrate to pH 7.4 with 1 N HCl or 4 M NaOH. Bring final volume to 500 ml. Yields a 1.5% solution of sodium salt.

APPENDIX B

AMMONIA ANALYSIS

Reagents

1) Phenol-Nitroprusside Solution

10 g Phenol

50 mg Sodium Nitroprusside

Dissolve in 1 liter with DD H_2O

2) Hypochlorite Solution

5 g Sodium Hydroxide

21.296 g Anhydrous Dibasic Sodium Phosphate (Na_2HPO_4) or 53.7 g Na_2HPO_4 * 12 H_2O

48 ml Clorox (5.25% NaclO) or 10 ml of Sodium Hypochlorite (10 - 14% Cl).

3) Sodium Tugstate (10%)

4) Sulfuric Acid (1 N)

5) Standard (Stock):

0.0471 g Ammonium sulfate in 1 liter of dH_2O

Stock solution contains 1000 ug NH_3-N per dl.

Preparation of Samples

1) Collect blood in a heparinized tube and centrifuge at 2 to 4° C for 10 minutes.

2) Transfer 1 ml of plasma to a 5 ml borosilicate glass tube containing 0.5 ml of tungstate.

3) Immediately add 0.5 ml of 1 N Sulfuric acid

4) Vortex thoroughly.

5) Centrifuge in a regrigerated unit at 1500 x G for 10 min.

6) Pipette 0.25 ml of protein-free supernatant into a 5 ml polystyrene tube (do in duplicate).

** Steps 7 and 8 are optional to same day continuation**

7) Cover and quick freeze using methanol-dry ice

8) Store at -70° C or dry ice for up to 48 h.

Standards

1) Individual standard solutions are made in 5 ml borosilicate glass tubes as follows:

Standard	ml Stock	ml H_2O
1000 ug/dl	1.000	0
750 ug/dl	0.750	0.250
500 ug/dl	0.500	0.500
300 ug/dl	0.300	0.700
250 ug/dl	0.250	0.750
200 ug/dl	0.200	0.800
100 ug/dl	0.100	0.900
50 ug/dl	0.050	0.950
BLANK	0.000	1.000

2) To each of the above tubes add 0.5 ml tungstate and then immediately add 0.5 ml of 1 N sulfuric acid.

3) Vortex and centrifuge in the same manner as the test samples.

4) In duplicate, pipette 0.25 ml of supernatant from each of the standards above into polystyrene tubes.

Colorimetric Reaction

1) To 0.25 ml of each supernatant, add 1.25 ml of phenol-nitroprusside solution and 1.25 ml of hypochlorite, vortexing after each addition.

2) Incubate at 37° C for 35 minutes (minimum). The color once developed is stable at room temperature for several hours.

3) Read absorbancy on a spectrophotometer at 625 nm.

4) Calculate concentrations of unknowns from a standard curve.

REFERENCE: McCullough, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clin. Chim. Acta 17:297-304.

APPENDIX C

UREA ANALYSIS

Urea Nitrogen kit from Sigma Diagnostics is based on the following reaction:

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

Reagents

1) Phenol Nitoprusside Solution
   Phenol, 50 g/L, sodium nitroprusside and stabilizer.
   Store in refrigerator at 2 - 6° C.

2) Alkaline Hypochlorite Solution
   Sodium hypochlorite, .2%, in alkali.
   Store in refrigerator at 2 - 6° C.

3) Urease Buffer Reagent
   Buffered urease from jack beans, 100 units, when prepared.

4) Urease Solution
   Prepared by reconstituting vial of Urease Buffer Reagent with 30 ml water. Stable at least 1 month if stored in refrigerator at 2 - 6° C.

5) Urea Nitrogen Standard Solution
   Urea at a urea N level of 30 mg/dl (10.7 mmol/l).

Sample Collection and Storage

Plasma: Add whole blood directly to a heparinized tube. Most anticoagulants will work, however, fluoride must not be used as it inhibits the urease reaction. Spin tubes for approximately 15 minutes and harvest plasma.

Procedure

1) Label test tubes in duplicate for standards, samples and blank.

2) To each, add 0.5 ml Urease Solution.

3) To blank, add 10 ul water.

   To standard add 10 ul of Urea Nitrogen Standard Solution.

To Sample, add 10 ul plasma.

4) Place tubes in a 37° water bath for 5 - 10 minutes, or let stand at room temperature for 15 - 20 minutes.

5) In the following order to each, add:

1.0 ml Phenol Nitroprusside Solution

1.0 ml Alkaline Hypochlorite Solution

5.0 ml water

Vortex after each addition.

6) Allow tubes to develop color at room termperature 20 - 30 minutes.

7) Read absorbance at 570 nm.

Calculations

$$\text{Plasma Urea N (mg/dl)} = \frac{\text{TEST}}{\text{STANDARD}} \times 30$$

Note

It may be advantageous to run a second set of tests on the same while eliminating the urease addition. This will test impurities picked up and corrects for ammonia nitrogen.

APPENDIX D

ALPHA-AMINO NITROGEN ANALYSIS

Reagents

1) 1 M $HClO_4$
2) 1 M NaOH
3) Sodium Borate Buffer
4) Picryl-sulfonic Acid
5) Crystalized Ornithine (as a Standard)

Procedure

1) Add .50 ml of plasma or standard
2) Add .50 ml 1 M $HClO_4$
3) Add 1 ml Water
4) Centrifuge at 3 g for 15 min.
5) Pipette .250 ml of supernatant into a 12 X 75 mm glass tube.
6) Add .100 ml 1 M NaOH
7) Add .500 ml Sodium Borate Buffer

****THE FOLLOWING STEPS MUST BE DONE IN NO LIGHT****

8) Add .500 ml Picryl-sulfonic Acid
9) Incubate in a water bath at 37° C for exactly 19 min.
10) Add .500 ml 1 N HCl
11) Let samples sit at room termperature for 5 min.
12) Read at 340 nm

Note

If dilution of sample with water is changed (before spinning) the addition of 1 M NaOH must be adjusted accordingly, so that following the

addition of the Sodium Borate Buffer the pH of the sample is between 8.5 and 9.2.

APPENDIX E

GLUCOSE ANALYSIS

Sigma Chemical Co. #510 enzymatic colorimetric in vitro determination for whole blood, plasma or serum is based upon the following coupled enzymatic reactions:

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + 2H_2O_2$$

$$H_2O_2 + \text{o-Dianisidine} \xrightarrow{\text{peroxidase}} \underset{\text{(colorless)}}{\text{oxidized o-Dianisidine}} \quad \underset{\text{(brown color)}}{}$$

The intesity of the brown color is measured at 450 nm and is proportional to the orginal glucose concentration.

Reagents

1) PGO Enzyme, Stock #510-6 (Preweighed capsules).

Contains glucose oxidase, peroxidase and buffer salts.

One capsule is diluted with double distilled (DD) H_2O to 100 ml and stored in an amber bottle at 0 to 5° C.

2) o-Dianisidine Dihydrochloride, Stock #510-50.

Preweighed vial is diluted with DD H_2O to 20 ml and stored at 0 to 5° C.

1.6 ml of this is added to 100 ml of PGO enzyme solution.

3) Glucose Standard solution, Stock #535-100.

Consists of a solution of beta-D-glucose, 100 mg/dl in benzoic acid, 0.1% and stored at 0 to 5° C.

4) 0.3 N Barium Hydroxide Solution, Stock #14-3.

5) 5.0% Zinc Sulfate Solution, Stock #14-4, $ZnSO_4 * 7\ H_2O$.

Sample Collection and Preparation

Blood samples should be collected in a container with a suitable anticoagulant. Immediately after sample is collected, pipette

0.2 ml blood into test tube containing 1.8 ml DD $_2$O, then vortex. To each tube add 1.0 ml barium hydroxide, vortex, then add 1.0 ml zinc sulfate and vortex again. Blank and standard are prepared in a similar manner except 0.2 ml of DD H_2O and 0.2 ml of glucose standard are used, respectively, in place of the blood. Tubes are then centrifuged at 3g for 15 min at 0 to 5° C.

Analysis

Prepare unknown, blank and standard tubes in duplicate as follows:

To each tube add 0.25 ml of supernatant from the respective centrifuged unknown, blank and standard tubes followed by 2.5 ml of PGO/color solution and vortex. Incubate all tubes at 37° C for 30 min in a waterbath.

Avoid direct exposure to sunlight or bright light. Following incubation, remove all tubes from waterbath. Read at 450 nm. Readings should be completed within 30 min.

Calculation

$$\text{Blood glucose (mg/dl)} = \frac{\text{Absorbance Unknown}}{\text{Absorbance Standard}} \times 100$$

APPENDIX F

FREE FATTY ACID ANALYSIS

NEFA kit from Wako and is based upon the following reactions:

FFA are activated to CoA ester by aceyl CoA sythetase (ACS).

$$R\text{-}COOH + ATP + CoA \xrightarrow{ACS} Acyl\text{-}CoA + AMP + PPi$$

Acyl-CoA is oxidized by acyl CoA oxidase (ACOD) to produce hydrogen peroxide.

$$Acyl\text{-}CoA + O_2 \xrightarrow{ACOD} 2,3\ trans\text{-}Enoyl\text{-}CoA + H_2O_2$$

Hydrogen peroxide is acted on by peroxidase (POD) in the presence of 3-methyl-N-ethyl-N-beta-hydroxyethyl-aniline (MEHA) and 4-aminoantipyrine (AAP) to form a product with a purple color which is meaured colorimetrically at 550 nm.

$$2H_2O_2 + MEHA + AAP \longrightarrow Purple\ Quinone\ Product + 4H_2O$$

Reagents

1) Color reagent "A". Each vial contains:

   Acyl-CoA synthetase
   Ascorbate oxidase
   CoA
   ATP
   4 Aminoantipyrine

2) Diluent for color reagent "A". Bottle contains:

   0.05 M Phosphate Buffer, pH 6.9
   Magnesium Sulfate
   Surfactant
   Stabilizers

3) Color reagent "B". Each vial contains:

   Acyl-CoA oxidase
   Peroxidase
   MEHA

4) Diluent for color reagent "B". Bottle contains:

Phenoxyethanol
Surfactant

5) NEFA standard. Each vial contains aqueous solution with:

1.0 mM Oleic acid
Surfactant
Stabilizers

Procedure

1) Preparation of standards:

Dilute the standard provided (1000 ueq/l) with double distilled H_2O to provide 125, 250 and 500 ueq/l standards. Validation tests show DD H_2O is an acceptable diluent.

2) Preparation of reagents:

Mix color reagent "A" by adding 10 ml of the specific "A" diluent to each vial. Gently invert the vial until contents are completely dissolved and then combine with 13.3 ml of DD H_2O.

Mix color reagent "B" by adding 20 ml of the specific "B" diluent per vial. Gently invert until contents are dissolved and then combine with 33.3 ml DD H_2O.

Solutions should be stored between 2 to 8° C and are stable for 5 d. Do not freeze solutions or expose them to direct sunlight.

Analysis

1) Assay each sample and standard in duplicate.

2) Add 0.4 ml DD H_2O to each tube.

3) Add 0.1 ml of serum, plasma or standard to each tube, followed by 0.35 ml of the combined reagent "A" plus H_2O mixture. Vortex and refigerate for up to 60 min if necessary.

4) Incubate tubes in a waterbath at 37° C for exactly 20 min.

5) Following incubation, add 0.8ml of the combined reagent "B" plus H_2O mixture to each tube, mix and incubate a second time at 37° C for exactly 20 min.

6) Remove tubes from waterbath, allow to equilibrate at room temperature for 5 min, then record to optical density at 550 nm using a blank prepared with reagents as zero.

(Volume in ml)

Tube	Standard	Plasma	Reagent "A" + H2O	Reagent "B" + H2O
Blank	--	--	0.35	0.80
Std in ueq/liter				
125	0.025	--	"	"
250	"	--	"	"
500	"	--	"	"
1000	"	--	"	"
UNK	---	0.10	0.35	0.80

Comments

1) Do not mix regents and supplies from test kits bearing different lot numbers.

2) The assay is sensitive to heparin use as a sample anticoagulant.

3) Samples which are hemolyzed may yield inaccurate results.

4) Ascorbic acid interferes with assay; therefore, ascorbate oxidase is included in reagent "A" mixture in the kit.

APPENDIX G

ACETOACETATE ANALYSIS

Treatment of Blood

1) Mix equal volumes of whole blood and 1 M $HCLO_4$ immediately after sampling.

2) Centrifuge at 3g for approximately 15 min.

3) Neutralize excess $HCLO_4$ with KOH so that the final pH is between 6.0 and 8.0.

4) Centrifuge off the potassium perchlorate formed, at approximately 3g for 15 min then pour off the supernatant.

5) Samples must be analyzed the day of collection.

Reagents

1) 0.1 M Phosphate Buffer (pH 6.8)

1.36 g KH_2PO_4 ---> 100 ml DD H_2O

1.74 g K_2HPO_4 ---> 100 ml DD H_2O

Mix equal volumes of each solution. Check pH and add the appropriate solution to bring the pH to 6.8.

2) NADH (approx. 1mM) (Disodium Salt) (Grade II, 98% Pure) (Boehringer Mannheim, cat. # 128023)

0.005 g NADH ---> 6.0 ml H_2O

Make up on day of use.

3) 3-Hydroxybutyrate Dehydrogenase (5.0 mg/ml) (Grade II) (Boehringer Mannheim, cat. # 127841)

4) 1.5 mM Acetoacetic Acid (ACAC) (Lithium Salt) (90 - 95% pure) (Sigma Chemical Co., cat. #A-8509)

0.01620 CH_3COCH_2COOH g ---> 100 ml H_2O

Make up on day of use.

Dilute to the following concentrations for the working standards: 0.15, 0.09, 0.075, 0.045, and 0.0225 mM.

Use two zero concentration standards as blanks.

The standards must be taken through the entire procedure as small amounts of perchlorate will affect enzyme activity.

Pipette into conical centrifuge tubs:

1.0 ml standard and 1.0 ml of 1 M $HCLO_4$.

Neutralize to pH 6.0 to 8.0 (Remember to record the volume required to adjust the pH !!!!)

Centrifuge at 3g for 15 minutes.

Standard dilutions:

A) 0.15 ---> 2.0 ml of 1.5 mM ACAC plus 18.0 ml H_2O

B) 0.09 ---> 12.0 ml of A plus 8.0 ml H_2O

C) 0.075 --> 4.0 ml of A plus 4.0 ml H_2O

D) 0.045 --> 10.0 ml of B plus 10.0 ml H_2O

E) 0.0225 -> 10.0 ml of D plus 10.0 ml H_2O

Analysis

Pipette into 12 X 75 mm culture tubes:

0.5 ml buffer

Sample/standard - 1.0 ml for fed sheep and 0.5 ml plus 0.5 ml H_2O for fasted sheep.

0.05 ml NADH

Read E_1 at 340 nm.

Add 0.005 ml 3-Hudroxybutyrate dehydrogenase and incubate at room temperature for approximately 20 min.

Read E_2 at 340 nm.

Important

NADH will "react" slowly at room temperature but at about the same rate in all samples. The zero concentration ACAC standards can be used as a control for this. Pipette NADH into all of the cuvettes at known time intervals, i.e., 15 to 60 s. Then read E_1 in all cuvettes at the same timed intervals. Incubate and read E_2 at same timed intervals.

Calculations

Calculate E_1 minus E_2 for all standards, samples and blanks:

Change in E of standard minus change in E of blank

Change in E of sample minus change in E of blank

Plot a standard cuve of change in E of standard versus concentration.

Read the concentration of the samples from the curve.

Multiply by appropriate dilution factors.

APPENDIX H

3-HYDROXYBUTYRATE ANALYSIS

Treatment of Blood

1) Mix equal volumes of whole blood and 1 M $HCLO_4$ immediately after sampling.

2) Centrifuge at 3g for approximately 15 min.

3) Neutralize excess $HCLO_4$ with KOH so that the final pH is between 6.0 and 8.0.

4) Centrifuge off the potassium perchlorate formed, at approximately 3g for 15 min then pour off the supernatant.

5) Samples may be stored at -20° C for one week.

Reagents

1) 0.1 M Tris-HCL Buffer (pH 8.5)

   2.42 g Tris ---> 50 ml DD H_2O

   pH ---> 8.5 with 1 M HCl

   Final volume ---> 200 ml

2) Hydrazine-Tris Buffer

   2.5 ml Hydrazine Hydrate

   0.05 g EDTA

   12.5 ml 1 M HCl

   Volume ---> 50 ml with Tris-HCL Buffer

   pH 8.5

   Make up on day of use.

3) 14 mM NAD^+ (Free Acid) (Grade II, 98% pure)
   (Boehringer Mannheim, cat. #127990)

   0.03 g NAD^+ ---> 3.0 ml DD H_2O

4) 3-Hydroxybutyrate Dehydrogenase (5 mg/ml)
   (Boehringer Mannheim, cat. #127841)

5) 2.0 mM 3-Hydroxybutyric Acids (Sodium Salt) (98% Pure) (Sigma Chemical Co., cat. #H-6501)

0.0656 $CH_3CHOHCH_2COOH$ g ---> 250 ml DD H_2O

Working standards:

2.0, 1.6, 1.2, 0.8 and 0.4 mM

The standards must go through the whole procedure and are treated as described in the acetoacetate assay.

Standards:

2.0 = 100% ---> 20.0 ml 2.0 mM BOHB plus 0.0 ml H_2O

1.6 = 80% ---> 16.0 ml 2.0 mM BOHB plus 4.0 ml H_2O

1.2 = 60% ---> 12.0 ml 2.0 mM BOHB plus 8.0 ml H_2O

0.8 = 40% ---> 8.0 ml 2.0 mM BOHB plus 12.0 ml H_2O

Analysis

Pipette into culture tubes:

Sample/Standard	0.25 ml (fed)	0.1 ml (fasted)
H_2O	0.75 ml	0.90 ml
Buffer	0.50 ml	0.50 ml
NAD^+	0.05 ml	0.05 ml

Read E_1 at 340 nm.

Add 0.005 ml 3-Hydroxybutyrate dehydrogenase and incubate at room temperature for about 45 min.

Read E_2 at 340 nm.

Important

NAD^+ and hydrozine form a complex which absorbs at 340 nm. Therefore, a slow constant increase in absorbance occurs. This is similar to the acetoacetate assay but in reverse. So pipette the NAD^+ and enzyme and take to absorbance readings at timed intervals as described to the acetoacetate assay.

Calculation

Same as for acetoacetate, with the exception of E is calculated by E_2 minus E_1.

APPENDIX I

VOLATILE FATTY ACID ANALYSIS

Reagents: Always use highest purity water!!!

H_2O/Triton - 20 ul Triton x-100 per 250 ml H_2O

1N NaOH - 40.0 g NaOH per liter

10 mM NaOH - Make stock 1 N NaOH (40 g/l) and dilute 5 ml to 500 ml each day of use.

1 N HCl - 83 ml per liter

Somogyi Filtrate

1) Zinc sulfate solution, 0.175 M (5%)
100 g $ZnSO_4$ * $7H_2O$ (reagent grade, unefforvesced); dissolve in distilled H_2O to make 2 liters.

2) Barium hydroxide, 0.15 M
95 g $Ba(OH)_2$ * $8H_2O$; dissolve in recently boiled and cooled distilled water to make 2 liters. Fairly stable if kept in stoppered and out of contact with air.

3) The actual concentrations of the 2 solutions are not so important, but they must exactly neutralize each other. To check:

- Pipette exactly 10 ml of zinc solution to a flask containing about 40 ml H_2O; add a few drops of phenolphthalein indicator (0.5 g/dl in 95% EtOH)

- Titrate with $Ba(OH)_2$ solution, slowly and with constant agitation.

- Titrate until 1 drop of $Ba(OH)_2$ solutions turns the titrated solutions a faint pink which lasts for 1 min.

The 10 ml of $ZnSO_4$ should require about 10 ml of $Ba(OH)_2$

If not equal strength, dilute or add additional $ZnSO_4$ to give equal strengths. Do not try and alter $Ba(OH)_2$.

Repeat the titration.

Keep solutions tightly stoppered - $Ba(OH)_2$ will have a white precipitate of $BaCO_3$ with exposure to air.

Standards

Stock	mw	Appoximate grams per liter	Approximate final concentration after dilution (mM)
Acetate	60.0	3.0	2.0
Propionate	74.0	0.9	0.5
Isobutyrate	88.1	0.4	0.2
Butyrate	88.1	0.8	0.4
2-Methyl butyrate	102.1	0.4	0.05
3-Methyl butyrate	102.1	-	0.10
Valerate	102.0	0.4	0.15
Lactate - Na^+	112.0	1.125	0.50
3-hydroxybutyrate	126.1	1.300	0.50

Stock Internal Standard

0.0363 g 2-ehtylbutryic acid (MW 116.16), qs to 100 ml H_2O

0.0481 g Pivalic acid, (MW 102.13), qs to 100 ml H_2O

Working Internal Standard (ISTD)

Add 4 ml each of stock. 2-ethylbutyrate and pivalic acid to 200 ml volumetric prior to use, qs to volume. Use 4 ml of the ISTD per sample.

Working Blood Standard

Add 4 ml stock blood standard, 4 ml of each stock internal standard to 100 ml volumetric. Add .92g Formic Acid, .378g oxalic acid, .0056g Na-lactate, .0063g 3-hydroxybutyrate and qs to volume. Make working standard with each set of samples, as you prepare the working standard.

Ion Exchange Columns

1) Materials:

AG 50W-X 8 - 100 - 200 mesh (Top Column)

BIO-REX 5 - 100 - 200 mesh (Bottom Column)

Polypropylene Econo - Column

2) Pack columns by preparing a slurry of resin in H_2O. Allow resin to settle and pour off fines 1 to 3 X.

3) Agitate slurry by swirling and pipette into columns,

Cation - 1.8 ml resin

Anion - .6 ml resin (May add slightly more)

4) Always leave columns overnight in H_2O. If allowed to sit overnight or longer, always leave water in the resin bed and plug the outlet.

Sample Treatment Through Columns

1) Using a pateur pipette stir-up each column immediately after unplugging the outley each morning. This will prevent air bubbles and promote an even flow through column. After H_2O has dripped through, place the cation column over the anion column.

2) Pour supernatant + ISTD into top of cation column, allow to drip through both columns, simultaneously and completely, NOTE: all of the supernatant will not go on to column at once.

3) Rince tubes 2x with 2 ml H_2O and pour into columns. Let each rinse drip through completely before adding the next.

4) Rince top (cation) column once with 2 ml H_2O.

5) Remove top column.

6) Rince bottom column with 2 ml H_2O.

7) Add 10 ml of 10 mM NaOH to bottom column and collect in 20 X 150 mm screw top tubes.

8) Shell freeze tubes and then freeze dry overnight.

9) Reconstitute with 1.0 ml of O-Phosphoric acid.
109 ml/l

10) Alternate method is same as above except for steps 7 and 8. Collect 10 ml of 10 mM NaOH in 50 ml beakers and oven dry overnight.

Regeneration of Columns

1) Top (Cation):
Fill reservoir (10 ml) with 1 M NaOH 1X (stir up resin with first NaOH)
Rinse 2 X with H_2O
Fill reservoir with 1 M HCl 2 X
Rinse 2 X with reservoir H_2O, till neutral effluent

2) Bottom (Anion):
Fill reservoir 2X with 1 N NaOH (stir up resin)
2X with H_2O

Chromotograhy

1) Column and Packing: 2 m by 2 mm glass column packed with 80/120 Carbopak B-DA 4% Carbowax 20 M

2) Carrier Gas: N_2, 24 ml/min. The column is maintained at 175° C, inlet and detector are 200° C.

3) Run time is 30 minutes.

VITA

Byron Clark Housewright was born in Fayetteville, Arkansas on October, 28 1965. Shortly thereafter the family moved to Abilene, Texas where he attended school through the second grade. In 1974 his family relocated to Stephenville, Texas where he completed his primary and secondary education. He graduated from Stephenville High School in May 1984.

He began a Bachelor of Science degree in Animal Science in the fall of 1984 at Texas Tech University. Graduating with a Bachelor of Science degree in 1988, he was accepted into graduate school at The University of Tennessee, Knoxville. Beginning the graduate program as a research assistant in the fall of 1988. He was awarded the Master of Science degree in Animal Science in August of 1990.

Immediately following the completion of the Master of Science degree he was accepted for the Ph.D. program at The University of Tennessee and began his studies toward the Doctor of Philosophy degree in September of 1990.