ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 7 (3): 223-233, 2008
ISSN 1682-8356

Examination of the Composition of the Luminal Fluid in the Small Intestine of Broilers and Absorption of Amino Acids under Various Ambient Temperatures Measured *In vivo*

M.A. Mitchell[1] and A. Lemme[2]
[1]Roslin Welfare Consultants, 12 Charlton Grove, Roslin, U.K (Former at Roslin Institute)
[2]Evonik - Degussa GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany

Abstract: Composition and normal concentrations of nutrients (glucose, methionine, sodium and potassium) as well as the pH and osmolarity were determined in digesta collected from different segments of the small intestine of broilers both under thermoneutral and heat stress conditions in order to formulate a perfusate closely simulating physiologically normal conditions of the small intestine. Whilst glucose and methionine levels of the luminal fluid decreased with passage ($p<0.05$) sodium and potassium increased ($p<0.05$). Heat stress (30°C) partly decreased concentrations of glucose and methionine in digesta ($p<0.05$). Based on these analyses, a luminal perfusate was composed and used to study the absorption of DL-methionine (DL-Met) and the liquid hydroxy analogue of methionine (DL-HMB) as well as of the DL-HMB di- and oligomers under different ambient temperature conditions *in vivo*. Adaptive responses on length and weight of small intestine (jejunum) as well as on absorption of both methionine sources were observed which were dependent on the magnitude of imposed heat load. At higher temperature (35°C) length and weight of the small intestine decreased while absorption rate of both Met sources increased compared to the thermoneutral condition (22°C). Data suggested higher absorption velocity for DL-Met compared to that for DL-HMB ($p>0.05$). There was no interaction between DL-Met and DL-HMB absorption and ambient temperature. It was demonstrated that only a marginal portion of the di-and oligomer fraction of DL-HMB was absorbed. Results suggest that the perfusate formulated on basis of luminal fluid analyses was suitable for investigating absorption of amino acids.

Key words: Broiler, luminal fluid, *in vivo* absorption, methionine sources

Introduction

The intestinal epithelium acts as the interface between the ingesta and the intercellular fluids and the circulation. Absorption of individual nutrients is mediated by a number of systems (Alpers, 1987; Hopfer, 1987). These include both active transporter mediated and passive mediated and non-mediated components (Pappenheimer, 1990; Buddington, 1992; Stevens, 1992; Sadowski and Medding, 1993; Karasov and Cork, 1994). Experimental determinations of nutrient transport or transfer rates and capacities are based upon exposure of intestinal tissue, cells or membrane preparations either *in vivo* or *in vitro* to a given range of substrate concentrations in the perfusates or incubation media. The basis for the selection of these concentrations is often unspecified and they are frequently matched to the characteristics of the transport component to be studied. Whilst this approach provides a sound mechanistic understanding of the individual transport systems and adaptations therein to physiological state, environmental challenge and altered dietary composition it is difficult to relate the findings to absorption in the whole animal during normal feeding. Indeed it has been proposed that "uncertainty about luminal concentration makes it difficult to calculate the relative contributions of mediated versus non-mediated transport from measures made *in vivo* or *in vitro*" (Karasov and Cork, 1994). A first step to achieving a more integrated model of whole animal absorption is therefore to measure the composition of the luminal fluid in the small intestine. This fluid not only contains the bulk phase concentrations of the nutrients to which the digestive/absorptive surface is exposed but represents a major determinant of the micro-environment in which digestion and absorption take place. More specifically the luminal fluid determines the ionic, pH and osmolarity micro-environment of the unstirred layer at the brush border membrane of active enterocytes. Surprisingly, few attempts have been made to measure comprehensively the luminal concentrations of substrates and electrolytes in the luminal bulk phase and those studies which do exist report either limited data or conflicting results, the latter being partly attributable to the different species studied and different locations of measurement within the small intestine (Cole, 1961; Nasset, 1964; Steiner and Gray, 1969; Nixon and Mawer, 1970; Mongin, 1976; Murakami *et al*., 1977; Ferraris *et al*., 1990; Savory and Mitchell, 1991; Karasov and Cork, 1994; Barfull *et al*., 2002).

There is an ongoing controversial debate on the bio-availability of methionine sources. Whilst the liquid hydroxy analogue of methionine (DL-HMB) has been reported to be only 65 to 70% available compared to DL-methionione (DL-Met) on "as is" basis (74-80% on molar basis) by Lemme *et al.* (2002, 2007) Sauer *et al.* (2007) and Jansman *et al.* (2003), other authors using a different approach report DL-HMB to be as bio-available as DL-Met (Vazquez-Anon *et al.*, 2006). In case of differences in bioavailability, differences in absorption efficiency might be a basic cause. Related research is also controversial. Whereas some scientists reported that there are different absorption mechanisms for the uptake of DL-Met (Na^+-dependent) and DL-HMB (H^+-dependent) and absorption by diffusion plays no role (Maenz and Engele-Schaan, 1996a,b; Martin-Venegas *et al.*, 2004) others believe that DL-HMB is mainly absorbed by diffusion (Knight and Dibner, 1984; Dibner *et al.*, 1988). Another controversy is about the availability of particularly the di-and oligomers of the DL-HMB which are reported to by hydrolyzed to monomers which are then efficiently absorbed (Martin-Venegas *et al.*, 2004) or which are reported to be detrimental for the bioavailability of total DL-HMB (Van Weerden *et al.*, 1992; Saunderson, 1991). So, absorption of methionine sources appeared to be an interesting example for studying absorption in an *in vivo* perfusion technique using a perfusate mimicking nutrient concentrations and other properties of digesta in a growing broiler.

The main objective of the present study was, therefore, to determine glucose, methionine (and DL-HMB), sodium and potassium concentrations and the pH and osmolarity of luminal fluid from different regions of the small intestine of broiler chickens. Comparisons of these parameters were made in birds maintained in thermoneutral conditions and groups exposed to chronic heat stress which is reported to alter absorption of nutrients including methionine sources as well as food intake and growth (Mitchell and Carlisle, 1992; Mitchell *et al.*, 1999; Dibner *et al.*, 1992). After development of a perfusate suitable for absorption examination, an *in vivo* perfusion methodology has been applied in order to study various aspects of absorption of two methionine sources in the jejunum-the part of the small intestine where the majority of amino acids is absorbed.

Materials and Methods

Determination of luminal fluid composition: Fourty eight day old female broiler chickens of a commercial strain were maintained in brooders until 21 days of age and fed a commercial starter diet being offered *ad libitum*. Ambient temperature was set at 31°C at day one and then reduced by 0.5 to 21°C. Birds were then transferred to a floor pen, littered with wood shavings, in a climate chamber controlled to 20±0.5°C and 50±8% relative humidity (mean water vapour density 9.2 gm^{-3}). These settings constituted control conditions. The birds were allowed *ad libitum* access to food and water. Throughout the experimental period they were fed a commercial broiler finisher formulation supplemented with either 0.15% DL-methionine or 0.17% DL-HMB (Table 1). The lighting cycle was 14 h light: 10 h darkness with the light period commencing at 06:00 h. At 28 days of age half the birds (12 DL-Met fed birds; 12 DL-HMB fed birds) were transferred to an adjacent, identical climate chamber maintained at 30±0.5°C and 60±8% relative humidity (mean water vapour density 18.2 gm^{-3}) designated heat stress. At that time body weight of birds was about 1200 g. The four groups were held under the control and heat stress conditions for a further 7 days. Alternate members of each group were then weighed and killed by intravenous administration of 200 mg per kg body weight of sodium pentobarbitone (Euthatal-Rhone-Merieux Ltd, UK). All birds were killed between 10:00 and 12:00 h. Upon cessation of respiration and all corneal and pupillary reflexes the abdominal skin and peritoneal wall were rapidly opened by a mid-line incision and the small intestine was ligated at both the gizzard-duodenal and ileo-caecal-colonic junctions using silk suture thread. The entire small intestine was quickly removed from the abdominal cavity and chilled by immersion in ice cold 0.9% saline. The intestine was then blotted dry and placed in a pre-chilled dissection tray. The duodenum was identified as extending from the sacchus dorsocranialis (antrum pyloricum) of the gizzard to the Ligament of Treitz at the flexura duodeno-jejunalis. The intestine was double ligated at this point. The jejunum is considered to extend from the end of the duodenum to Diverticulum Vitelli (Meckels Diverticulum). Another double ligature was placed at this point. The remainder of the small intestine was regarded as the ileum. Each sealed segment thus identified was isolated by cutting between the double ligatures and then opened by an incision along its whole length on the ante-mesenteric border. Samples of the digesta (solid and liquid) were removed by gentle scraping with a plastic spatula from three 4 cm sampling locations in each segment. These sites were 2.5 cm from the top and bottom of each segment and one in the middle. The samples from each segment were combined in a chilled glass tubes and were rapidly centrifuged at 3500 G at 0°C for 10 min. The supernatant was divided into 3 aliquots. One aliquot was placed in a glass tube, treated with an equal volume of 12% perchloric acid, mixed thoroughly, allowed to stand for 5 min and then centrifuged at 4000 G for 5 min. A second untreated aliquot was subjected to immediate analysis for sodium, potassium, pH and osmolarity. The remaining supernatant was frozen at-20°C pending analysis for glucose.

Methionine and DL-HMB concentrations were determined by HPLC analysis in the luminal fluid

Table 1: Ingredients and analyzed nutrient content of the basal experimental grower diets

Ingredients (g/kg)		Energy (MJ ME/kg) and analyzed nutrient content (g/kg)	
Wheat	560	Energy (MJ ME/kg)	12.98
Barley	100	Crude protein	206
Soybean meal	198	Methionine[1]	4.6
Fish meal	40	TSAA[2]	7.8
Meat and bone meal	50	Calcium	10.0
Vegetable oil	40	Phosphorus	7.0
Limestone	2.6	Sodium	1.5
Salt	2.5	Chloride	2.3
Lysine HCl	0.3		
Vitamin premix	2.5		
Mineral premix	2.5		
Choline chloride (50%)	0.3		

[1]to this diet either 1.5g/kg DL-Met or 1.7g/kg DL-HMB were added; [2]TSAA: Total sulphur amino acids (methionine plus cysteine)

supernatants. The perchloric acid treated sample supernatant (second centrifugation) was diluted 1:1 with pure water and filtered through a 0.45 µm PVDF-D syringe filter (Millipore Ltd. U.K.) in to HPLC sampling tubes. Stock standard solutions of 5 mM DL-Met and DL-HMB as HMB calcium salt were prepared in pure water. DL-HMB-Ca consist exclusively of monomers. Serial dilutions were employed to yield a standard range down to 0.1 mM. HPLC analysis was performed on the aqueous digesta supernatants and perfusates using a Waters 840 controller fitted with WISP 710 B injection system, two Waters 510 pumps and a model 490 programmable multi-wavelength detector set to 0.500 AUFS (Millipore U.K Ltd.-Waters Chromatography Division, U.K.). The column packing consisted of a Waters mBondapack C18 RP (3.9×3000 mm) with a C18 column guard. Column temperature was maintained at 40°C. A two solvent, mobile phase, gradient method of elution was employed. Solvent A contained 0.05 M orthophosphoric acid (pH 3.5) and solvent B was a mixture of 70%:30% 0.05 M orthophosphoric acid and HPLC grade acetonitrile (Rathburns Ltd, U.K.). Injection volume of the stock standard solutions was 20 µL. Methionine eluted at approximately 7.5 min while DL-HMB-Ca eluted at 15.4 min. The same system was also applied to determine the concentrations of the liquid DL-HMB including the monomers and the associated oligomers, mainly dimmers. DL-HMB monomer again eluted at 15.3 min with the dimmer-peak at 19.5 min. Each sample was run for 30 min to allow re - equilibration of the column. Calibrations were performed at the beginning and end of every batch of samples analyzed. Peak identification and integration was performed upon a PC based system using Waters Expert Software (Waters Ltd. U.K.).

The pH of the untreated luminal fluid supernatants (sample volume 40 µL) was determined directly using a clinical blood gas analyzer (Model 238-CIBA-Corning, Halstead, UK), with body temperature correction. Sodium and potassium concentrations were also measured on these samples with a Na^+/K^+ clinical analyzer (Model 614-CIBA-Corning, Halstead, UK). This system is based upon a potentiometric ion sensitive electrode. Samples were diluted according to a urine analysis protocol (CIBA-Corning, Halstead, U.K.) and read against slope standards for sodium (100 mM) and potassium (120 mM). Osmolarity of the fresh supernatant was determined by freezing point depression. A 200 µL aliquot was introduced in to an Advanced Digimatic Osmometer (Model 3DII-Advanced Instruments, Massachusetts, USA) in a sample cup. The samples were read against sodium chloride standards of 100 and 900 mOsmoles per kg of water.

The concentration of glucose in the luminal fluid supernatant was measured using a commercially available kit based on the glucose oxidase principle (Wako Glucose C; Alpha Laboratories Ltd., Hampshire, UK) modified for use in an automated plate reading spectrophotometer (Titertek 2, Autoflow Laboratories, UK).

Absorption of methionine sources: For comparing absorption of DL-Met and DL-HMB (free acid) as well as of DL-HMB (free acid) and DL-HMB-Ca and the *in vivo* perfusate was formulated (Table 4) on the basis of the analysis of the luminal fluid composition. The concentrations of Na and K, DL-Met (10 mM) and DL-HMB (10 mM) and glucose as well as the pH and osmolarity were selected to best reflect the average conditions prevailing in the broiler small intestine. Total osmolarity was adjusted by addition of mannitol. All perfusates were freshly prepared in pure water on the day of perfusion. Control perfusate was analyzed on every experimental day. The 72 broilers used for this investigation were of same origin and were kept under same conditions as described above. In addition, a third temperature regime simulating severe heat stress (35°C, 70% rel. humidity) was employed. At this time deep body temperatures were determined by insertion of a rectal thermistor probe 5 cm through the cloaca. The birds were anaesthetized using halothane as described by Mitchell and Carlisle (1992). A mid-line incision was made attaining access to the peritoneal cavity with minimal haemorrhage. Then careful dissection of the

body wall, muscle layers and intra-abdominal fat pads followed. The duodenal loop containing the pancreas was reflected allowing access to a 20 cm segment of central jejunum which was exteriorized. The section employed extended, in an oral direction, from flexura duodenojejunales to 15 cm above Meckel's diverticulum. The duodenum was returned to the peritoneal cavity and the jejunal segment with intact vascular and nerve supplies was cut at the proximal and distal ends and washed free of digesta with warm 0.9% saline and finally cannulated. The cannulae were connected by silicon tubing to a perfusion circuit to establish an aboral flow. A peristaltic pump pumped the perfusate through a heating coil supplied from a heated circulator pump which maintained the solutions in the proximal cannula at 41.5°C. After passage through the segment the perfusate was returned to the reservoir for re-circulation. Perfusion rate was 4 ml/min and each perfusion lasted for 30 min. Initial volume of the perfusate was 25 mL. On completion of the surgical preparation the perfused loop was surrounded by the dissected sub-cutaneous tissues and skin and was covered with cotton wool soaked in pre-warmed physiological saline. The whole bird was covered by insulation and placed on a controlled heating pad in order that body temperature might be regulated to the pre-operative level during perfusion. At the end of the 30 min-period the outlet of the reservoir was disconnected to terminate the perfusion and all the solution was returned to the reservoir. Weighing of the perfusate before and after perfusion allowed estimation of fluid transfer. Substrate uptake was measured by analysis and comparison of the pro and post-perfusion solutions. Each animal was used for only one perfusion. The animals were killed by intravenous injection of sodium pentobarbitone, the perfused jejunal loops were rapidly but carefully cut free from the rest of the intestine at the pints of cannulation, removed and dissected free of mesentery, blood vessels and fat. The unstretched length of the segment was determined and the wet weight of the tissue measured. Dry tissue weight was obtained following drying to constant weight in and oven at 80°C. Uptakes of substrate have been calculated and expressed on the basis of both tissue length and weight. DL-Met and DL-HMB absorption was determined in birds receiving the corresponding dietary methionine source in each of the environmental temperature treatments.

Absorption of monomers and dimmers of DL-HMB compared to monomeric DL-HMB-Ca: In a second trial 24 birds of the same origin, kept under the same conditions as described above were utilized. In order to investigate the absorption of monomers and dimers either a perfusate with DL-HMB containing both fractions or a perfusate with the calcium salt of DL-HMB (DL-HMB-Ca) containing only monomers was used. The same *in vivo* luminal perfusion technique as described above was applied.

Where appropriate, the data are presented as the means±one standard error of the mean (SEM). Data were analyzed by standard procedures (ANOVA) procedures of SAS (SAS Institute, 1998). Treatment differences between treatments were tested for significance by using unpaired Students t-test.

Results

Determination of luminal fluid composition: The compositions of luminal fluids in duodenum, jejunum and ileum for control and heat stress groups are presented in Table 2. Glucose concentration exhibited a marked proximal-distal negative gradient in both thermal treatments, the highest values being found in the upper small intestine. There were no significant differences in glucose concentration between duodenum and jejunum but the ileal concentration was lower ($p<0.05$) than in the other two segments at both 20 and 30°C. The only significant difference in glucose concentration associated with the thermal environments was a 25% lower value in the jejunum of the heat stressed group ($p<0.05$).

Luminal fluid sodium concentration ranged from approximately 67-118 mM across segments and treatments. In the control birds the concentrations in duodenum and jejunum were identical but also did not differ significantly from the numerically higher value observed in the ileum. The sodium concentration was significantly higher (75%) in the duodenum of heat stressed birds than in controls ($p<0.05$) but no environmental temperature associated significant differences were observed in the other two segments. There was a tendency towards a proximal-distal negative gradient for sodium concentration in the heat stress group the duodenal value being significantly greater than those in the other two segments ($p<0.05$).

Potassium concentration in the luminal bulk phase was highest in the ileum in both control and heat stress birds. The latter group exhibiting a 38% higher value ($p<0.05$) than their thermoneutral counterparts. In contrast duodenal potassium was 62% higher ($p<0.05$) in the control group. The duodenal value was also significantly higher (49%) than the jejunal in the thermoneutral birds ($p<0.05$).

Luminal pH increased from proximal to distal small intestine. There were no significant differences in the mean duodenal and jejunal values but a significant alkalinization in the ileum in both groups ($p<0.05$).

The osmolarity of the luminal fluid ranged from 342-463 mOsm across groups and segments. The values were consistently higher in the heat stressed birds, the apparent increases being approximately 20% in duodenum and ileum ($p<0.05$). In the control group

Table 2: Luminal concentration of glucose, methionine, sodium, potassium and supernatant pH and osmolarity in the duodenum, jejunum and ileum of control (thermoneutral, 20°C) and heat stressed (30°C) broiler chickens (values are presented as the mean±one SEM: n = 12)

	Temperature regime	Duodenum	Jejunum	Ileum
Glucose	Control	68.1±6.4	58.9±4.0	7.6±1.6
(mmol/litre)	Heat stress	56.1±6.8	44.4±3.3	12.4±2.4
Sodium	Control	67.3±5.0	68.5±4.0	83.5±6.5
(mmol/litre)	Heat stress	118.4±2.8	83.2±7.6	71.3±4.0
Potassium	Control	19.8±1.3	13.3±1.0	26.8±2.4
(mmol/litre)	Heat stress	12.2±0.5	14.5±1.4	37.1±3.0
pH	Control	6.4±0.06	6.6±0.16	7.7±0.09
	Heat stress	6.6±0.06	6.8±0.16	8.2±0.26
Osmolarity	Control	389±16.7	430±7.8	342±4.9
(mOsm/litre)	Heat stress	463±6.9	452±22.2	413±13.6

Statistical significances ($p<0.05$) by unpaired Students t-test are described in "Results"

Table 3: Luminal concentration of methionine and DL-2-hydroxy-2-methylthiobutanoic acid (DL-HMB) in the duodenum, jejunum and ileum of broilers fed a 0.15% or 0.17% supplemented DL-Met or DL-HMB diet under control (thermoneutral, 22°C) and heat stress (30°C) conditions (n = 12)

Dietary treatment		DL-Met*	DL-HMB*	DL-Met*	DL-HMB*
Temperature		22°C	22°C	30°C	30°C
Duodenum (mmol/100ml)	Methionine	10.98±1.01	10.56±0.97	1.86±0.36	5.09±0.94
Jejunum (mmol/100ml)	Methionine	6.86±0.55	4.17±0.60	1.25±0.15	4.60±0.27
Ileum (mmol/100ml)	Methionine	2.96±0.47	2.65±0.21	1.26±0.08	3.67±0.52
Duodenum (mmol/100ml)	DL-HMB	-	5.55±0.80	-	1.33±0.37
Jejunum (mmol/100ml)	DL-HMB	-	2.09±0.45	-	1.44±0.25
Ileum (mmol/100ml)	DL-HMB	-	3.60±0.93	-	1.55±0.52

Statistical significances ($p<0.05$) by unpaired Students t-test are described in "Results"

the highest osmolarity was found in the jejunum ($p<0.05$) whereas no significant gradient occurred in the heat stress birds.

Bulk phase methionine and methionine hydroxy analogue also exhibited a proximal-distal negative gradient in the thermoneutral group (Table 3). The highest values (11.0 mM/10.6 mM) in the duodenum being 60% (DL-Met) or even 153% (DL-HMB) higher than that in the jejunum ($p<0.05$), which in turn was 2.3 or 1.6 fold greater than the ileal value ($p<0.05$). There were no significant differences between methionine sources. The concentrations of methionine in all three intestinal segments in the heat stress group were significantly lower than the corresponding values in the control group ($p<0.05$) and being reduced to less than 1.9 mM. Methionine levels measured of the DL-HMB fed birds tended to be higher in all three segments. Whilst luminal DL-HMB concentration decreased from 5.6 to 3.6 mM with proximal-distal passage in the thermoneutral group, concentrations were low in all intestinal segments in the heat stressed group ranging between 1.3 and 1.6 mM.

Absorption of methionine sources: Results on the investigation of absorption of supplemental DL-Met and DL-HMB are presented in Table 5. Exposure to heat stress did influence body weight development. Whilst moderate heat stress (30°C) had little effect the higher heat load induced a 20 and 16% reduction in body weight in the DL-Met and DL-HMB supplemented birds, respectively ($p<0.05$).

Control body temperatures were the same in birds receiving the two supplements. Moderate heat stress treatment resulted in increased rectal temperatures ($p<0.05$). Highest heat load resulted in a further significant increase in deep body temperatures ($p<0.05$). Methionine sources did affect dry weight of jejunum neither under control nor under heat stress conditions. Dry weight was significantly decreased by moderate heat stress in the DL-HMB birds (-15%) and by severe heat stress in both the DL-Met (-39%) and DL-HMB (-43%) supplemented groups ($p<0.05$).

There was no significant difference in the absorption of DL-Met and DL-HMB under either temperature regime although an apparent numerical difference due to methionine source was observed with DL-Met uptake exceeding that of DL-HMB by 23%. Moderate heat stress (30°C) significantly reduced uptake per unit length in both DL-Met (-35%) and DL-HMB (-29%) fed groups ($p<0.05$). Contrastingly, the more severe heat stress appeared to increase absorption per unit length for both DL-Met (+44%) and DL-HMB (+55%) although this only achieved statistical significance in the latter case ($p<0.05$). This differential response in absorption in relation to the magnitude of heat load imposed and the resultant heat stress was further reflected in the jejunal uptakes of each of the two substrates expressed per unit dry weight of intestinal tissue. The greater absorption of DL-Met compared to that of DL-HMB failed to achieve significance. The highest heat load, however, induced profound increases in the uptake of both methionine

sources compared to control values and even larger effects relative to the absorption per unit weight in the moderate heat stress group ($p<0.05$).

Absorption of monomers and dimers of DL-HMB compared to monomeric DL-HMB-Ca: Results of the examination of the uptake of monomers and di-and oligomers of DL-HMB compared to monomeric DL-HMBCa are presented in Table 6. There were no differences in body weight between the DL-HMB and DL-HMB-Ca treatment and were thus averaged. Birds of the heat stress treatments were about 100 g lighter ($p>0.05$). At 32°C the rectal temperature increased by 1.0°C ($p<0.05$) compared to 0.7°C at the lower heat load of the first experiment (see above) indicating that this response may be on the border proposed between moderate and severe heat stress. Dry weight of jejunum decreased significantly by 25% due to the imposed heat stress ($p<0.05$). The reductions in absorption per unit length of both the free acid form and the calcium salt at 32°C compared to thermoneutral controls were of the same order as those observed with the previous moderate heat stress treatment (Table 5) but did not reach statistical significance. Also, no significant differences for the total uptake both related to the unit jejunum length and related to the unit jejunal dry weight were found between DL-HMB and DL-HMB-Ca. Dimer absorption constituted only 1-2% of total absorption and was not influenced by ambient temperature.

Discussion

Determination of luminal fluid composition: It should be emphasized that the mean values for the concentrations of luminal substrates, ions and pH and osmolarity calculated in the present study are based upon fluid samples from upper, middle and lower sections of each intestinal segment. As a consequence this geometric mean may mask higher and lower values of each parameter in the extreme regions of the intestine e.g. upper duodenum and lower ileum. It is proposed, however, that the values are a useful guide to the average luminal fluid composition in each region of the small intestine. The values obtained for glucose concentration in the proximal regions (45-68 mM) are generally consistent with those previously reported in other strains of domestic fowl (52.5 mM-Barfull *et al.*, 2002) as are the proximal distal gradient values (34.0-8.0 mM-Savory and Mitchell, 1991). It is proposed that glucose absorption via the SGLT-1 system alters the properties of the tight junctions between enterocytes (Atisook *et al.*, 1990; Turner, 2000; Nusrat *et al.*, 2000) thus increasing the transport of glucose through the passive paracellular pathway, which may then make a substantial contribution to total glucose absorption (Atisook *et al.*, 1990; Pappenheimer, 1998). This effect would be greater at elevated luminal glucose concentrations. In parallel it is suggested that glucose activated recruitment of GLUT-2 transporters to the brush border membrane (Kellett and Helliwell, 2000) will also result in increased facilitated uptake in the presence of high luminal glucose. It is therefore necessary to consider the available luminal substrate concentration when assessing total absorption *in vivo* or in intact tissue *in vitro*. For example assessing absorptive capacity of the intestine by measuring uptake at luminal concentrations consistent with recognized kinetic characteristics of the transport systems may underestimate the true potential for substrate absorption by a stimulated paracellular route at concentrations prevailing under normal feeding conditions. Thus the relationship between luminal glucose concentration and the importance of paracellular uptake allows integration of data relating to mechanisms of absorption and nutritionally relevant uptake capacities and adaptations therein. It has been suggested that in a nectarivorous bird and in man 80-90% of glucose uptake is by the paracellular pathway (Karasov and Cork, 1994; Pappenheimer, 1998). It should be noted that absolute substrate concentrations at specific apical membrane transport sites may be different to the bulk phase concentrations considered in the present study due to the presence of an unstirred layer of water (Ferraris *et al.*, 1990). In the current study there was little effect of heat stress upon the actual glucose concentrations in each intestinal segment or the proximal-distal gradient. It might be suggested that a tendency towards a lower glucose in the upper small intestine may reflect reduced food intake during heat stress.

There have been few studies examining the ionic composition of luminal fluid in the intestine of the domestic fowl. The present findings indicate a solution with a lower sodium concentration and a higher potassium concentration than plasma. The values for sodium concentration are very similar to those reported by Mongin (1976) for the laying hen whilst the current values for potassium are about half those described in each intestinal segment by the same author. In the current control group there appears to be a higher cation content in the ileal fluid than in the upper small intestine. A proximal-distal negative gradient for sodium was observed in the heat stress group and the duodenal sodium concentration was elevated during hyperthermia. Potassium concentrations were higher in the distal small intestine a difference exacerbated by heat stress. The most striking feature of the ionic composition is the clear and substantial differences from avian plasma and physiological buffers used to study absorption of individual substrates. This is also true for pH and osmolarity and it must be questioned if more physiological solutions, closer in composition to luminal fluid, should be employed as perfusion media in *in vivo* studies. The pH values found in the present

study reveal a marked acidity in the upper small intestine and an alkalinization in the ileum. All the values are substantially at variance with the recognized physiological pH of 7.38. In this context it is interesting that Wiseman (1974) reported that the optimum absorptive activity of several amino acid transport systems occurs at "non-physiological pH. The pH optima were in fact close to those reported herein for luminal fluid.

The luminal fluid is hyper osmolar throughout the small intestine compared to plasma and the degree is increased during heat stress. The luminal fluid of the upper segments tends to have a higher osmolarity than that of the ileum. This is in part due to the absorption of the osmotically active substrates such as sugars and amino acids in the duodenum and jejunum but with some compensation via the higher sodium and potassium concentrations. Indeed if it is assumed that sodium and potassium will be accompanied by comparable concentrations of chloride then the proportion of the total osmolarity accounted for by glucose, methionine and the electrolytes would be 65% in the duodenum and 0.68 in the ileum of the control birds. The degree of hyper osmolarity (+100 to 150 mOsm) is in accordance with the values reported by Ferraris *et al.* (1990) for rats, rabbits and dogs. It was suggested that a greater degree of hyper osmolarity could not be sustained in the face of the small intestines high osmotic permeability to water. The increased osmolarity observed in the heat stressed birds may result from altered water balance in response to the prolonged, moderate hyperthermia.

Whilst only methionine has been considered in the present study it may be speculated that general concentrations and distributions along the intestine should be similar for all amino acids. Methionine and DL-HMB were selected as they are often subject of study in poultry nutrition and adaptations in its absorption in response to heat stress have been previously described (Mitchell and Carlisle, 1992; Dibner *et al.*, 1992). Previous studies in mammals have indicated that methionine concentrations in luminal fluid are lower than most other amino acids but generally the concentration is only 2-3 fold higher for the most abundant peptide (Nasset, 1964; Steiner and Gray, 1969; Nixon and Mawer, 1970). These authors reported methionine concentrations ranging from 100 µM to approximately 1.0 mM in rodent and human small intestine. The comparably high range of luminal methionine concentrations found in the present study may reflect the high protein content of poultry feedstuffs and the routine supplementation of commercial diets with this amino acid (1.5 g methionine per kg diet in this case) or its hydroxy analogue. However, strong decrease of luminal methionine concentrations and, less pronounced DL-HMB concentrations from duodenum to ileum clearly indicate that both bulk of free and protein bound amino acids are absorbed in the upper part of small intestine. As for glucose it is important to incorporate knowledge of luminal substrate concentration into experimental designs where an understanding of physiologically meaningful uptakes is sought. Thus *in vivo* or *in vitro* studies where the intestinal mucosa is exposed to very high non-physiological concentrations of amino acids and where passive absorption may predominate, may lead to erroneous interpretation of the findings. Such studies examining the uptake of methionine sources in chicken intestine *in vitro* at a luminal concentration of 50 mM have been reported (Dibner *et al.*, 1992). The marked reduction in luminal methionine and DL-HMB in the present study in response to heat stress may reflect a reduced food intake and delivery of amino acids to the small intestine during hyperthermia or an altered absorptive pattern. An apparent effect of DL-HMB supplementation is that of raising the luminal methionine concentration particularly in heat stressed birds compared to those receiving the DL-Met supplement. It might be concluded that this reflects an interaction at the level of absorptive process resulting in a decreased uptake of protein bound methionine. However, the mechanism for this phenomenon is unknown.

In summary the luminal fluid of the small intestine of the domestic fowl may be described as a hyper osmolar, low sodium, high potassium solution of non-physiological pH. The associated concentrations of major nutrients vary significantly with anatomical location and may differ substantially from those assumed from known characteristics of transport systems or used in many experimental protocols. It is apparent that the composition of luminal fluid in the small intestine has important implications for the design of physiologically meaningful nutrient absorption studies and their interpretation in relation to absorptive, mechanisms, capacities and adaptations.

Based on these findings a perfusate for *in vivo* absorption studies using a loop of jejunum was formulated (Table 4). The concentrations of sodium and potassium and glucose and the pH and osmolarity were selected to best reflect the average conditions prevailing in the broiler small intestine during post-prandial phase. Moreover, based upon the findings on amino acid concentrations and the available knowledge of the kinetics and possible routes of absorption of the two methionine sources a perfusate substrate concentration of 10 mM was chosen for both methionine sources. Whilst normal luminal concentrations of each methionine source may vary between 1 and 11 mM, it is not possible to undertake valid scientific comparisons of *in vivo* uptake with different luminal concentrations of each substrate for different treatments.

Table 4: Composition of the perfusate according to the findings of the digesta analysis

Nutrient, criteria	
Sodium chloride, mmol/100ml	100
Potassium chloride, mmol/100ml	10
Glucose, mmol/100ml	50
Mannitol, mmol/100ml	120
Methionine, mmol/100ml	10
pH	6.6
Total osmolarity, mOsm/100ml	400

Table 5: The effects of chronic moderate (30°C) and severe (35°C) heat stress on jejunal absorption in birds receiving diets equivalently supplemented with DL-methionine (DL-Met) or DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMB) expressed both per unit of jejunum length and per unit dry weight of jejunum (n=12)

	DL-Met	DL-HMB
Body weights at 28 days (g)*		
22°C	1225±32	1183±31
30°C	1110±75	1176±48
35°C	982±26	998±31
Deep body temperature (°C)*		
22°C	41.3±0.2	41.2±0.2
30°C	41.6±0.4	41.9±0.7
35°C	42.8±0.6	42.7±0.8
Dry weight of jejunum (g/cm)*		
22°C	0.076±0.004	0.085±0.004
30°C	0.076±0.007	0.072±0.004
35°C	0.046±0.003	0.048±0.001
Absorption per unit jejunum length (nmoles/cm/min)*		
22°C	99±12.6	75±7.3
30°C	64±3.9	53±8.8
35°C	141±21.6	116±14.5
Absorption per unit jejunal dry weight (nmol/g/min)*		
22°C	1342±209	949±118
30°C	982±104	817±104
35°C	3160±570	2454±320

Statistical significances ($p<0.05$) by unpaired Students t-test are described in "Results"

Absorption of methionine sources: Whereas body temperature was only marginally increased at an environmental temperature of 30°C a profound and identical degree of hyperthermia (+1.5°C) was induced in birds receiving both methionine sources at the higher heat load (35°C) suggesting no major difference in heat production or dissipation between the dietary treatments. Possibly as a result of the physiological stress, heat stress induced markedly different responses in growth and intestinal tissue weights. Reduced growth rate during heat stress is a consequence of the adaptive decrease in food intake and changes in endocrine function (Fuller and Dale, 1979; Mitchell *et al*., 1994). Interestingly, whilst the moderate heat stress produced no significant effects upon body weight and intestinal weights in DL-Met or DL-HMB fed birds, approximately 16-20% decreases in final body weight and 40% reductions in both wet and dry small intestine tissue weights were associated with exposure to the higher temperature regime being consistent with previously published data (Mitchell and Carlisle, 1992). It might be suggested that during the severe heat stress, less intestinal tissue has thus to maintain more body mass, despite the concomitant decrease in growth rate and that some adaptation in absorptive function is therefore essential. However, since there were no differences between dietary treatments it is proposed that it was appropriate to determine and compare absorption of the two methionine sources on the bases of intestinal length and tissue segment weight.

The jejunal absorption of DL-Met of 98.3 nmol/cm/min or 1342 nmol/g/min measured in the thermoneutral birds (Table 5) were both consistent with those reported by Mitchell and Carlisle (1992). Whilst there were no statistically significant differences between DL-Met and DL-HMB uptake, the absorption of DL-HMB appeared to be only 76-83% (nmoles/cm/min) or 71-83% (nmoles/g/min) as high as that of DL-Met being consistent with the findings of others: Maenz and Engele-Schaan (1996a) reported a higher affinity and rate of the DL-Met carrier (Na^+-dependent) compared to the H^+-dependent one responsible for DL-HMB uptake. In their studies absorption by diffusion was of minor importance compared to active transport mechanisms (Maenz and Engele-Schaan, 1996b). In addition, Brachet and Puigserver (1989) concluded that when comparing the kinetic parameters of DL-Met and DL-HMB absorption, the existences of a higher capacity carrier and of a faster diffusion process were "undoubtedly in favour for the amino acid relative to the hydroxy analogue". Moreover, research of Drew *et al*. (2003) revealed that under conventional conditions disappearance of a substantial portion of monomeric L-HMB in the digesta is due to microbial degradation in the small intestine. But even under germ-free conditions, which can be compared to the current study in which the effect of microbial activity was avoided by washing the intestine with saline, a small but significant higher absorption was observed for L-Met compared to L-HMB. This difference developed with transit through the small intestine but was significant already in the jejunum (Drew *et al*., 2003). It could be argued that due to the lower velocity and capacity of the DL-HMB absorption mechanism and the fast passage of the digesta through the small intestine in broilers, quantitative intestinal DL-HMB uptake is smaller compared to the DL-Met uptake. DL-Met and DL-HMB uptake per unit length increased significantly by around 50% at the highest heat load-on a tissue weight basis the corresponding increases were 2.5 fold compared to the thermoneutral treatments (Table 5). As was previously proposed (Mitchell and Carlisle, 1992), these increases in substrate absorption observed during exposure to a temperature of 35°C may be an adaptive strategy to optimize nutrient absorption

Table 6: The effects of chronic moderate (32°C) heat stress on body weight, jejunal dry weight as well as on jejunal absorption, expressed both per unit of jejunum length and per unit dry weight of jejunum, in birds receiving diets equivalently supplemented with DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMB-FA) and DL-2-hydroxy-4-methylthiobutanoic calcium salt (DL-HMB-Ca)

	DL-HMB-Ca	DL-HMB-FA Monomers	DL-HMB-FA Dimers	DL-HMB-FA Total (Mono-plus Dimers)
body weight (28 days, g)*				
22°C		1154±32		
32°C		1055±43		
Deep body temperature*				
22°C		41.2±0.3		
32°C		42.2±0.6		
dry weight of jejunum (g/cm)*				
22°C		0.081±0.005		
32°C		0.061±0.009		
Absorption**	---------- per unit jejunum length (nmol/cm/min) ----------			
22°C	65.2±12.6	66.4±11.6	1.0±0.3	68.5±12.0
32°C	42.6±4.5	53.2±7.3	1.1±0.1	55.3±8.0
Absorption**	---------- per unit jejunal dry weight (nmol/g/min) ----------			
22°C	751±89	895±168	13±9	922±175
32°C	789±99	928±210	17±6	961±231

* n = 12 broilers; ** n = 6 broilers per treatment; Statistical significances ($p<0.05$) by unpaired Students t-test are described in "Results"

and efficiency in response to reduced feed intake. This may be achieved from a smaller absorptive compartment, an anatomical response which in itself will reduce energy and amino acid utilization and requirement as the normal intestinal mucosa with its inherent rapid cell division (Secor *et al*., 1994). Turnover constitutes a major consumer of these resources. The capacity of the small intestine for such adaptive responses involving alterations in the carrier systems of the enterocyte and the regulation of mucosal mass is well recognized and has been extensively described (i.e. Jankowski *et al*., 1994; Jenkins and Thompson, 1994). However, although ambient temperature affected DL-Met and DL-HMB absorption as such there was no beneficial effect found for DL-HMB absorption being in line to findings of Maenz and Engele-Schaan (1996a,b) but in contrast to results of Knight *et al*. (1994) and Dibner *et al*. (1992).

Absorption of monomers and dimers of DL-HMB compared to monomeric DL-HMB-Ca: Different biological efficiencies have been ascribed to the calcium salt and free acid of DL-HMB (Boebel and Baker, 1982; Van Weerden *et al*., 1982). The presence of polymeric or oligomeric forms of DL-HMB in the free acid preparation may account for this phenomenon, at least in part, through less effective utilisation and increased urinary excretion of the polymers (Saunderson, 1991). Whilst the dimeric and trimeric forms of the DL-HMB may be converted to the monomer by acid hydrolysis (Martin-Venegas *et al*., 2004), this process is very slow under physiological conditions (Koban and Koberstein, 1984). It may be assumed that the oligomers present in the diet will be unchanged and at similar levels in the digesta in the small intestine and thus available for absorption. The complementary experiment comparing the absorption of DL-HMB as the calcium salt (DL-HMB-Ca) and the free acid (DL-HMB) under thermoneutral (22°C) and heat stress conditions (32°C) indicated that 18.6% of DL-HMB existed in oligomeric form mainly as dimer being in close agreement with the findings of Koban and Koberstein (1984). It may be argued, however, that if the dimer is absorbed, then the total molar DL-HMB uptake should be quantified as monomer uptake plus 2×dimer uptake. This principle has been applied (Table 6). Accordingly, dimer absorption constituted only 1-2% of total DL-HMB-FA uptake. No significant differences in uptake velocity of DL-HMB-Ca and DL-HMB were found at either temperature. Responses to the temperature regime were exactly intermediate to those observed at 30 and 35°C which may reflect the initiation of the adaptations in absorptive function of the intestinal epithelium. Comparing the proportion of di-and oligomers in DL-HMB-FA (18.6%) with the same proportion of absorbed fractions (1-2%), poor uptake of the di-and oligomers becomes obvious. This effect occurred at both temperature regimes suggesting, that di-and oligomers are absorbed only to a small extent. This in turn raises the question about the conclusion from an experiment from Saunderson (1991) stating a substantial renal excretion of non-monomeric DL-HMB molecules. Such an excretion, however, would suggest a substantial uptake of that DL-HMB fraction. A recent study using physiological luminal concentrations of DL-HMB has reported that HMB oligomers are as efficiently absorbed as HMB monomers by the small intestine of the fowl (Martin-Venegas *et al*., 2004). This was concluded from HMB monomer serosal appearance and appearance in the intestinal wall tissue of various segments of the small intestine after feeding commercial DL-HMB and a product consisting only of monomers. However, as HMB has to be converted to L-Met, appearance of methionine in serum after application of commercial HMB (unfortunately not of the

monomeric product) has been measured. These processes were most pronounced at a low luminal pH of 5.5 suggesting that HMB is rapidly absorbed by an H^+ dependent process throughout the small intestine (Martin-Venegas *et al.*, 2004). It was not investigated whether the effects described in that study are affected by chronic heat stress.

In conclusion, luminal fluid of broiler chickens being fed common type of diet has been examined and based on the findings a perfusate which can be used for absorption studies has been developed. The perfusate with a pH of 6.6 and DL-Met and DL-HMB concentrations of 10 mM and thus designed to precisely simulate normal physiological conditions was applied in an *in vivo* investigation (intestinal loop technique). In this broiler trial the absorption of different methionine sources was studied *in vivo* at different ambient temperatures. The findings indicate adaptive responses in the absorption of the two methionine sources by the small intestine induced by chronic heat stress. At higher temperature (35°C) length and weight of the small intestine were decreased while absorption of both Met sources was higher compared to conditions at 22°C. Although statistically not significant, the absorption rates for DL-methionine were always higher than those for DL-HMB under both thermoneutral and heat stress conditions. Moreover, in a second trial it was demonstrated that the di-and oligomer fraction of DL-HMB was absorbed only to a small extent.

References

Alpers, D.H., 1987. Digestion and absorption of carbohydrates and proteins. In: Johnson, L.R. (Ed), Physiology of the Gastrointestinal Tract, Second Edition, Raven Press, New York, pp: 1469-1488.

Atisook, K., S. Carlson and J.L. Madara, 1990. Effects of phloridzin and sodium on glucose-elicited alterations of cell junctions in intestinal epithelia. Am. J. Physiol., 258: C77-C85.

Barfull, A., C. Garriga, M. Mitjans and J.M. Planas, 2002. Ontogenetic expression and regulation of Na^+-D-Glucose cotransporter in jejunum of domestic chicken. Am. J. Physiol., 282: G559-G564.

Boebel, K.P. and D.H. Baker, 1982. Efficacy of the calcium salt and free acid forms of methionine hydroxy analogue for chicks. Poult. Sci., 61: 1167-1175.

Brachet, P. and A. Puigserver, 1989. Na+-independent and nonstereospecific transport of 2-hydroxy 4-methylthiobutanoic acid by brush border membrane vesicles from chick small intestine. Comp. Biochem. Physiol., 94B: 157-163.

Buddington, R.K., 1992. Intestinal nutrient transport during ontogeny of vertebrates. Am. J. Physiol., 263: R503-R509.

Cole, A.S., 1961. Soluble material in the gastrointestinal tract of rats under normal feeding conditions. Nature 191, July 29th, pp: 502-503.

Dibner, J.J., C.A. Atwell and F.J. Ivey, 1992. Effect of heat stress on 2-hydroxy-4-(methylthio)butanoic acid and DL-methionine absorption measured in vitro. Poult. Sci., 71: 1900-1910.

Dibner, J.J., C.D. Knight, R.A. Swick and F.J. Ivey, 1988. Absorption of 14C-2-hydroxy-4-(methylthio)butanoic acid (Alimet®) from the hindgut of the broiler chick. Poult. Sci., 67: 1314-1321.

Drew, M.D., A.G. van Kessel and D.D. Maenz, 2003. Absorption of methionine and 2-hydroxy-4-methylthiobutoanic acid in conventional and germ-free chickens. Poult. Sci., 82: 1149-1153.

Ferraris, R.P., K.C. Sasan Yasharpour, K. Lloyd, R. Mirzayan and M. Diamond, 1990. Luminal glucose concentrations in the gut under normal conditions. Am. J. Physiol., 259: G822-G837.

Fuller, H.L. and N.M. Dale, 1979. Effect of diet on heat stress in broilers. Proceedings of the Georgia Nutrition Conference, Athens: 56.

Hopfer, U., 1987. Membrane transport mechanisms for hexoses and amino acids in the small intestine. In: Johnson, L.R. (Ed), Physiology of the Gastrointestinal Tract, Second Edition, Raven Press, New York, pp: 1499-1527.

Jankowski, J.A., R.A. Goodlad and N.A. Wright, 1994. Maintenance of normal intestinal mucosa: function, structure and adaptation. Gut. suppl., 1: S1-S4.

Jansman, A.J.M., C.A. Kan and J. Wiebenga, 2003. Comparison of the biological efficacy of DL-methionine and hydroxy-4-methylthiobutanoic acid (HMB) in pigs and poultry. CVB documentation report No. 29, Central Bureau for Livestock Feeding (CVB), Lelystad, The Netherlands.

Jenkins, A.P. and R.P.H. Thompson, 1994. Mechanisms of small intestinal adaptation. Dig. Dis., 12: 15-27.

Karasov, W.H. and S.J. Cork, 1994. Glucose absorption by a nectarivous bird: the passive pathway is paramount. Am. J. Physiol., 267: G18-G26.

Kellett, G.L. and P.A. Helliwell, 2000. The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. Biochem. J., 350: 155-162.

Knight, C.D. and J.J. Dibner, 1984. Comparative absorption of 2-hydroxy-4-(methylthio)-butanoic acid and L-methionine in the broiler chick. J. Nutr., 114: 2179-2186.

Knight, C.D., C.W. Wuelling, C.A. Atwell and J.J. Dibner, 1994. Effect of intermittent periods of high environmental temperature on broiler performance responses to sources of methionine activity. Poult. Sci., 73: 627-639.

Koban, H.G. and E. Koberstein, 1984. Kinetics of hydrolysis of dimeric and trimeric methionine hydroxy analogue free acid under physiological conditions of pH and temperature. J. Agric. Food Chem., 32: 393-396.

Lemme, A., A. Petri and M. Redshaw, 2007. What's new about commercial methionine sources in poultry. Degussa AminoNews™-Review: 1-35.

Lemme, A., D. Hoehler, J.J. Brennan and P.F. Mannion, 2002. Relative effectiveness of methionine hydroxy analogue compared to DL-methionine in broiler chickens. Poult. Sci., 81: 838-845.

Maenz, D.D. and C.M. Engele-Schaan, 1996a. Methionine and 2-hydroxy-4-methylthiobutanoic acid are transported by distinct Na^+-dependent and H^+-dependent systems in the brush border membrane of the chick intestinal epithelium. J. Nutr., 126: 529-536.

Maenz, D.D. and C.M. Engele-Schaan, 1996b. Methionine and 2-hydroxy-4-methylthiobutanoic acid are partially converted to nonabsorbed compounds during passage through the small intestine and heat exposure does not affect small intestinal absorption of methionine sources in broiler chicks. J. Nutr., 126: 1438-1444.

Martin-Venegas, R., J.F. Soriano-Garcia, M.P. Virnadell, P.A. Geraert and R. Ferrer, 2004. Oligomers are not the limiting factor in absorption of DL-2-hydroxy-4 (methylthio) butanoic acid in the chicken small intestine. Poult. Sci., 85: 56-63.

Mitchell, M.A., P. Kan and C. Goddard, 1994. The regulation of thyrotrophic function during chronic heat stress in the domestic fowl. Proceedings of the 17th Conf. Eu. Comp. Endocrinologists, 5-10 Sep., Cordoba, Spain, 83.

Mitchell, M.A. and A.J. Carlisle, 1992. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (gallus domesticus). Comp. Biochem. Physiol., 101A: 137-142.

Mitchell, M.A., A.J. Carlisle and R.R. Hunter, 1999. Cellular mechanisms of adaptation in intestinal hexose absorption in the broiler chicken during chronic heat stress. Poult. Sci., 78 (supplement 1): 56, 248.

Mongin, P., 1976. Ionic constituents and osmolality of the small intestinal fluids of the laying hen. Br. Poult. Sci., 17: 383-392.

Murakami, E., M. Saito and M. Suda, 1977. Contribution of diffusive pathway in intestinal absorption of glucose in rat under normal feeding condition. Experientia, 33: 1469.

Nasset, E.S., 1964. The role of the digestive tract in protein metabolism. Am. J. Dig. Dis. New Series, 9: 175-190.

Nixon, S.E. and G.E. Mawer, 1970. The digestion and absorption of protein in man. Br. J. Nutr., 24: 241-258.

Nusrat, A., J.R. Turner and J.L. Madara, 2000. Molecular physiology and pathophysiology of tight junctions IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines and immune cells. Am. J. Physiol., 279: G851-G857.

Pappenheimer, J.R., 1990. Paracellular intestinal absorption of glucose, creatinine and mannitol in normal animals: relation to body size. Am. J. Physiol., 259: G290-G299.

Pappenheimer, J.R., 1998. Scaling of dimensions of small intestines in non-ruminant eutherian mammals and its significance for absorptive mechanisms. Comp. Biochem. Physiol., 121A: 45-58.

Sadowski, D.C. and J.B. Medding, 1993. Luminal nutrients alter tight-junction permeability in the rat jejunum: an *in vivo* perfusion model. Can. J. Physiol. Pharmacol., 71: 835-839.

SAS Institute, 1998. SAS user's guide: Statistics. Version 7.0 edition. SAS Institute. Inc., Cary, NC.

Sauer, N., K. Emrich, H.P. Piepho, A. Lemme, M.S. Redshaw and R. Mosenthin, 2007. Meta-Analysis on the relative effectiveness of the liquid hydroxy analogue of methionine compared with DL-Met in broilers using multi-exponential regression. Proceedings 16th European Symposium on Poultry Nutrition, August 26-30, Strasbourg, 227-230 (full paper in preparation).

Saunderson, C.L., 1991. Metabolism of methionine and its nutritional analogues. Poult. Int. November: 34-38.

Savory, C.J. and M.A. Mitchell, 1991. Absorption of hexose and pentose sugars *in vivo* in perfused intestinal segments in the fowl. Comp. Biochem. Physiol., 101A: 969-974.

Secor, S.M., E.D. Stein and J.M. Diamond, 1994. Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. Am. J. Physiol., 266: G695-G705.

Steiner, M. and S.J. Gray, 1969. Effect of starvation on intestinal amino acid absorption. Am. J. Physiol., 217: 747-752.

Stevens, B.R., 1992. Vertebrate intestine apical membrane mechanisms of organic nutrient transport. Am. J. Physiol., 263: R458-R463.

Turner, J.R., 2000. Show me the pathway! Regulation of paracellular permeability by Na^+-glucose cotransport. Adv. Drug Delivery Rev., 41: 265-281.

Van Weerden, E.J., H.L. Bertram and J.B. Schutte, 1982. Comparison of DL-methionine, DL-methionine-Na, DL-methionine hydroxy analogue-Ca and DL-methionine hydroxy analogue free acid in broilers by using a crystalline amino acid diet. Poult. Sci., 61: 1125-1130.

Van Weerden, E.J., J.B. Schutte and H.L. Bertram, 1992. Utilisation of the polymers of methionine hydroxy analog free acid (MHA-FA) in broiler chicks. Arch. Geflügelk., 56: 63-68.

Wiseman, G., 1974. Absorption of protein digestion products. In: Smyth, D.H. (Ed), Biomembranes, Volume 4A, Plenum Press, London, pp: 363-481.