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Discover Animals

RESEARCH

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Effect of sweet potato leaf silage as a protein source on growth performance, physiological and serum biochemical response of growing pigs under moderate heat stress

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Abstract
In the context of climate change, feeding pigs with agricultural co-products could reduce the carbon footprint of pig production and improve pig response to Heat Stress (HS). The aims of the present experiment were: (1) to investigate the effects of replacing 70% of the dietary crude protein (CP) by sweet potato (SP) leaves silage on growth performance, slaughter traits and serum biochemical response and (2) to evaluate the effect of this diet on heat tolerance compared to a diet based on soybean meal (SBM). Forty-eight Large White barrows were allocated to two diets differing in their protein source. The silage diet (SIL) was formulated with a protein source based mainly on local resources: SP silage replacing 70% of SBM and supplemented with 3 synthetic AA, DL-methionine, L-Lysine and L-Threonine, while the protein source of the control diet (CON) was 100% SBM. Within each diet, pigs were allocated to two environmental treatments, Thermoneutral (TN) vs. Heat Stress (HS). Results showed that irrespective of temperature, the SIL diet reduced daily feed intake and increased nitrogen excretion. Total protein, blood urea nitrogen and albumin in the serum were reduced in the SIL diet, confirming protein metabolism changes. However, average daily gain and carcass weight were unaffected by soybean CP replacement. There was no effect of temperature or interaction diet x temperature on performance. Nevertheless, thermoregulatory parameters were reduced in the SIL diet, suggesting lower heat production when replacing soybean CP by SP silage.

Keywords Agricultural co-products, Growing pigs, Tropical climate, Carcass traits, Thermoregulation, Blood parameters

1 Background

Approximately 55% of the carbon footprint of pig production comes from feed and this is mainly due to the inclusion of soybean meal (SBM) as a major source of protein in the diet [1]. The high carbon footprint of SBM is partly due to large transportation distances but most importantly to Land Use Change such as deforestation in South America [2]. Therefore, the environmental impact of pig production could be reduced by replacing SBM by protein-rich agricultural co-products. In tropical and subtropical climates, which represent around 50% of the world pig production [3], a variety of alternative farming systems co-exist along with the intensive model. Among these smallholder systems, mixed crop-livestock systems promote a close integration of crops and animal production, including the use of local feed resources available on the farm [4]. Many of the local resources are rich in energy but low in protein (banana, sugar cane…). However, different types of foliage are rich in protein and available in large quantity, such as cassava leaves (*Manihot esculenta*) or sweet potato (SP) leaves (*Ipomoea batatas*). The use of forage plants as an alternative protein resource for sustainable pig production in the tropics has been reviewed by Kambashi et al. [5]. Sweet-potato (SP) foliage (vines and leaves) protein content ranges between 16 and 29% [5] and the protein has an amino acid profile close to the ideal protein for pigs, except for Methionine and Lysine [6–8]. These forages can be used fresh, dry or ensiled. The ensiling technique is useful to ensure longer availability of the resources on the farm than at the time of harvest.

Chronic Heat stress (HS) concerns most of the tropical regions, at least during some part of the year. Because pig lack functional sweat glands, they are particularly sensitive to HS. During HS, pigs reduce their voluntary feed intake to decrease their metabolic heat production. Therefore, HS have direct and indirect effects that negatively impacts growth and carcass quality, but also sow reproduction and overall animal welfare. Studies in growing pig showed that HS also damages the intestinal barrier and downregulates the expression of certain amino acids (AA) transporters in the gut epithelium, therefore reducing the digestibility of some AA [9, 10]. Combined with the decrease in feed intake, there is less AA available for growth. Supplementation of the diet with 20% of DL-methionine has been shown to reduce the damage of HS to the intestinal barrier effect and to improve growth under HS [11]. Interestingly, supplementation with 20% DL-methionine and L-Lysine to ensiled cassava leaf silage in Vietnam also lead to increased pig performance compared to un-supplemented silage [12].

Based on these studies, in the present assay, we chose to formulate a diet with a protein source based mainly on local resources: SP silage replacing 70% of SBM and supplemented with 3 synthetic AA, DL-methionine, L-Lysine and L-Threonine, which was compared to a diet which protein source was 100% SBM. The objectives were: (1) to investigate the effects of SP silage on growth performance, slaughter traits and serum biochemical response, (2) to evaluate the effect of sweet potato silage on heat tolerance compared to SBM. The hypotheses were that : (1) SBM could be partly replaced by SP silage without impacting growth performance and carcass traits, (2) a diet based on SP silage and supplemented with free AA could improve pig heat stress response.

2 Materials and methods

2.1 Silage preparation

Sweet potatoes foliage (leaves and vines) were harvested in local farms in Guadeloupe, French West Indies (latitude 168 N, longitude 618 W) in February and November 2022, at the time of tuber harvest. The foliage was left overnight for wilting to reduce moisture content, before being chopped into 2–3 cm long pieces with a grinder. Chopped leaves were mixed with diluted sugar cane molasses (1/5 dilution in water) to activate the fermentation process (10% of the wilted weight of foliage) [6]. The mixture was then kept in 35 L plastic containers (approximately 25 kg of silage) and sealed hermetically. The silage was stored in the sealed containers at room temperature for a minimum of 2 months and a maximum of 10 months before use. At the time of opening, silage was inspected visually for any traces of mold contamination; if mold was observed, the barrel was discarded. The quality of the different containers of sweet potato leaf silage was monitored by measuring the pH upon opening. The mean pH of the silage was 4.3 ± 0.5. The ensiled sweet potato foliage was fed to pigs for a maximum of 2 days after opening a container.

2.2 Animals and experiment design

All experimental protocols were approved by the Animal Care and Use Committee of French West Indies and Guyana (#69–2012-2) and the French Ministry of Agriculture authorized the study under the reference APAFIS#6070-2016070721289156v3 on living animals at the INRAE facility under the direction of N. Minatchy (INRAE-PTEA).

A total of 48 Large White barrows (43.6 ± 0.8 kg) were used in 2 replicates on INRAE experimental facilities (PTEA, Duclos, Guadeloupe, French West Indies, https://doi.org/10.17180/50N1-KN86*)*. All the animals used in this study were produced by INRAE PTEA experimental facilities. The experimental design consisted of two environmental treatments, Thermoneutral (TN) vs. Heat Stress (HS), and within each environment, two diets differing in their protein source.

The thermoneutrality (TN) condition consisted in an indoor room with a ventilated system allowing a constant temperature (21.5 °C ± 0.05 °C) whereas the heat stress (HS) condition consisted of an outdoor room following the environmental temperature and humidity fluctuations corresponding to the warm season of the tropical climate, between 20.3 °C and 27.9 °C.

The control diet (CON) was composed of green banana (*ad libitum*) and SBM, whereas in the silage diet, 70% of the CP from soybean was replaced by ensiled SP foliage, supplemented with 3 synthetic amino acids, DL-methionine, L-Lysine and L-Threonine. Therefore, there were 4 experimental groups: TN-CON, TN-SIL, HS-CON, HS-SIL. Within each replicate, pigs were allotted to one of the four experimental groups according to live Body Weight (BW) and litter origin.

At 14 weeks of age, animals were placed in individual metal-slatted pens (1.60 × 0.84 m) equipped with a stainless-steel feeder and a nipple water drinker. Animals could see, smell and hear each other to maintain a maximum of social interactions. The experiment started when the pigs reached 17 weeks of age, three weeks after batching so that the pigs could adapt to their new environment and diet, and lasted for 28 days.

For each environmental condition, pigs were offered the 2 different diets: half of the pigs (6 pigs/room) received the CON diet and the other half received the SIL diet. The CON diet was formulated at 168 g of CP/kg of DM, which corresponds to the CP needs of a 30 kg growing pigs [13]. The SIL diet was lower in protein (99 g of CP/kg of DM) due to the low CP level of the SP silage but was supplemented with free AA. The energy part of both diets was constituted of *ad libitum* green banana, which is a good energy source for pigs [14, 15]. Detailed composition of diets and chemical composition of ingredients are shown in Tables 1 and 2, respectively.

Table 1 Dry matter (g per kg) and chemical composition (g per kg of DM) of the ingredients used to formulate diets

Ingredient chemical composition	Green banana	SP leaf silage	Soybean meal
DM, g/kg	172	113	854
Crude Protein, g/kg DM	47	111	470
Organic matter, g/kg DM	946	832	927
NDF, g/kg DM	200	447	314
ADF, g/kg DM	69	362	74
ADL, g/kg DM	31	116	5

NDF: Neutral Detergent Fibers, ADF: Acidic Detergent Fibers, ADL: Acidic Detergent Lignin; ME: Metabolisable Energy

Table 2 Ingredient content (% of DM), dry matter (g per kg) and chemical composition (g per kg of DM) of diets

Item	CON	SIL
Ingredient content, **% DM**		
Green banana	59	51
Sweet potatoe silage	–	36
Soybean meal	41	13
L-lysine	–	0.3
D-L méthionine	–	0.1
L-thréonine	–	0.2
Chemical composition		
DM, g/kg	255	160
Crude Protein, g/kg DM	168	99
Organic matter, g/kg DM	815	915
NDF, g/kg DM	214	275
ADF, g/kg DM	62	177
ADL, g/kg DM	17	59

CON: Control Diet, SIL: Silage diet supplemented with Lysin, methionine and threonine, NDF: Neutral Detergent Fibers, ADF: Acidic Detergent Fibers, ADL: Acidic Detergent Lignin

2.3 Feeding

During their pre-experimental growth, the pigs were fed a conventional diet as pellet that met the nutritional requirements of LW growing pigs according to standard recommendations [13], with corn, wheat middling, and soybean meal, and containing 13.53 MJ of DE, 164 g/kg of CP.

A transition diet was started 12 days before the beginning of the experiment: for 3 days, the animals received a feed ration of 75% pellets and 25% bananas, then for 5 days, their diet was 50% pellets and 50% bananas. Three days before the beginning of the trial, all the animals were fed the experimental diet, i.e. 25% of the portion in SBM and 75% of bananas for the CON group in each building, or 30% of the portion in sweet potato silage and the rest with bananas, SBM and synthetic amino acids for the SIL group.

Meals were distributed at different times of the day: (1) from 6:00 to 8:00 am, half of the SBM ration (CON) or SP silage with half of the SBM and the synthetic AA (SIL), (2) from 9:00 to 11:00 am, green bananas for all the groups, and (3) from 12:00 to 14:00 pm, the rest of the protein ration. Banana was distributed *ad libitum*, starting with a 2 kg/animal during the adaptation period and adjusting the quantity everyday depending on refusals. For each animal, refusals were collected separately for each ingredients of the feed, before the next distribution and pooled per animal and ingredient the following morning.

Water was available to all pigs *ad libitum* from a nipple drinker designed to limit waste. During the 2 weeks of digestibility (at the beginning and end of the trial), pens were reduced in size (0.94 × 0.50 m) to allow collection of urine and faeces from each animal.

2.4 Measurements

2.4.1 Growth and slaughter

Ambient temperature and humidity were monitored continuously in both rooms using a stand-alone USB data logger (EL-USB-2+; DATAQ Instruments, Inc., Akron, OH) placed at an extremity of each room (one measurement every 10 min).

Body weight (BW) of pigs was measured at the beginning of the experiment (D0), then at the beginning of each week (D7, D14, D21) and before slaughter (D28 or D29).

The individual daily feed intake (FI) of the pigs was measured as the difference between offered and refused feed. Refusals and spillages were collected daily before the first feed distribution of the day and used to correct FI on an as-fed basis. Refusal samples of the different feed ingredients (bananas, silage, SBM) were taken every day and pooled individually at the end of each week for dry matter (DM) determination (96 h at 65 °C). Feed samples were taken at the time of diet preparation and were pooled for each week for DM determination.

Digestibility was measured during two seven-day collection periods, during the first and last week of the experiment. During these collection periods, total faeces and urine were collected daily, weighted and stored at 4 °C until the end of the collection period. Sulphuric acid (0.1 N; 10%, v/v) was added to the daily urine in order to avoid ammonia losses during collection and storage. At the end of the collection period, faeces and urine were homogenized and samples were taken for chemical analysis: two samples of faeces were heat dried (96 h at 65 °C) for DM determination and one was freeze-dried for the laboratory analyses [16].

Pigs were slaughtered in the experimental slaughterhouse of INRAE-PTEA by electrical stunning and exsanguination and in compliance with the current EU regulations (Commission Delegated Regulation, 2017). Hot carcass, perirenal fat, and visceral organs (liver, kidneys, and heart) were weighed just after slaughter.

2.4.2 Physiological measurements

Skin temperature (ST) and rectal temperature (RT) were measured during weighing, before the first meal (at 0800 h) with a thermocouple probe thermometer (type K, model 88002 K-IEC; Omega Engineering Inc., Stamford, CT) and a digital thermometer (Microlife Corp., Paris, France), respectively. Respiratory rate (RR) was visually estimated by counting the flank movements of resting pigs for one minute, once a week at different times of the day (9:00, 11:00 or 14:00). In addition, 7 days before the start of the experiment, a measurement probe for measuring internal body temperature (Anipill®, BodyCap, Caen, France) was implanted on all the pigs. The capsule was implanted 2 to 3 cm deep into the brachiocephalic muscle at the neck of the animal, following the methods described in Renaudeau et al. [17]. Healing at incision site, as well as animal behaviour, were monitored closely during the days post-implantation. All the animals fully recovered from the procedure and probe recording could be supervised throughout the experiment using the monitoring system. Probes recorded body temperature at the muscular level (Tmuscle) every 15 min during the 4 weeks of the experiments.

Blood samples were collected on restrained animals by venipuncture from the jugular vein with a 5 ml BD vacutainers ® tube with EDTA (ethylene diamine tetra-acetic acid) on days 1 and 22 at 0800 h before the animals received their meal. Blood was centrifuged at 3,400 × g for 10 min at 4 °C to obtain plasma and was then stored at – 20 °C.

2.4.3 *Chemical analysis*

Feed and faeces samples were milled through a one-mm screen prior to analysis. Organic matter (OM) and nitrogen (N) of the feed ingredients were performed according to AOAC [18]. Dry faecal samples were analysed for DM and N and fresh samples of urine for N by the same methods as for the feeds. Cell wall components [neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL)] in feed and excreta samples were determined as described by Van Soest et al. [19] by using a sequential procedure with prior amylolytic treatment.

2.5 Serum biochemical assay

One plasma aliquot was analysed for a routine blood panel consisting of Blood Urea Nitrogen (BUN), creatinine, glucose, total protein, albumin, creatine kinase, amylase, Alanine Transaminase (ALT), Aspartate Transaminase (AST), alkaline phosphatase, γ-glutamyl transferase (GGT), phosphorus and cholesterol. All plasma samples were analysed on an M-Scan II chemistry analyser (Melet Schloesing Laboratoires, Osny, France).

2.6 Calculations and statistical analysis

A temperature–humidity index (THI) was calculated for each day based on the following formula [20] : THI = 0.8 + T +(RH/100)*(T-14.4) + 46.4, in which T is the average daily T (°C) and RH is the average relative humidity.

The results were submitted to an analysis of variance (linear mixed model) to evaluate the effects of ambient temperature ($n = 2$), dietary treatment ($n = 2$), and the interaction between temperature and diet on the dependent variable. The model also included the fixed effect of replicate ($n = 2$) and a random intercept for each individual animal to account for repeated measures within subjects. For digestibility data, as well as thermoregulatory measured (ST, RT, and RR), the week of measurement and its interaction with other relevant effects were added as fixed effects. For RR, the hour of measurement and its interaction with other relevant effects were added as fixed effects.

Statistical analyses were performed using R software (version 4.4.1), using the *nlme* package [21] for linear mixed-effects modelling, and the *emmeans* package [22] for estimated marginal means and post-hoc comparisons.

The circadian rhythm of Tmuscle was studied by grouping together measurements for each hourly interval (from 0000 to 2400 h) for each pig. Data was then submitted to an analysis of variance (linear mixed model) using the *nlme* package [21] in R with the fixed effects of hour (24 h), week ($n = 4$), replicate ($n = 2$), temperature ($n = 2$), diet ($n = 2$) and the following interactions : temperature x diet, hour × diet, hour × temperature, and hour × temperature × diet. Animal was specified as random effect and a compound symmetry (CS) correlation structure was specified to model the within-subject correlation of repeated measurements over time.

Data are reported as least squares means computed with the *emmeans* package and are considered significant if $p \leq 0.05$.

3 Results

3.1 Climatic characteristics

Figure 1 shows the variation of hourly ambient temperature, relative humidity and THI in the two rooms of the experimental facility. When comparing the 2 environments, the difference in average hourly temperature between the TN and HS rooms was of 2.84 °C. In the controlled temperature room, corresponding to the TN environment, daily temperature stayed constant at 21.5 °C ± 0.05 °C, with an average humidity of 86.1 ± 1%. In the HS treatment where T and RH were not controlled by a climatizing system, conditions in the semi-open room followed the outside climatic change corresponding to the warm season of the tropical climate. The average ambient temperature ranged from 20.3 °C (at 0700 h) to 27.9 °C (at 1300 h) and the relative humidity varied between 68.4 and 97.2%. The hourly fluctuation of ambient temperature showed that the minimum and maximum values were reached at 0700 h (20.3 °C) and at 1300 h (27.9 °C). Contrary to ambient temperature, relative humidity was greatest at about 0700 h and lowest at 1300 h (i.e., 97.2 and 68.4%, respectively).

3.2 Composition of diet, nitrogen metabolism and digestibility

Diet with SP silage was lower in CP, but had a higher fibre and lignin content than the CON diet (Table 1). Effect of diet and temperature on nitrogen (N) metabolism and

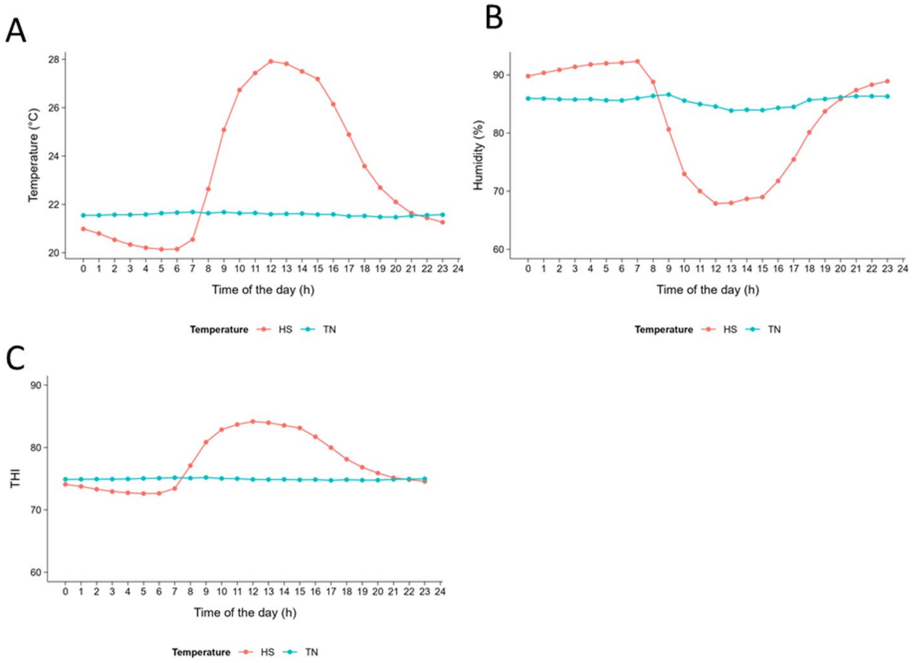

Fig. 1 Hourly climatic variation in the facilities according to the conditions (HS: Heat Stress, TN: Thermoneutral). THI: Temperature Humidity Index, calculated with the following formula [20] : THI = 0.8 + T +(RH/100)*(T-14.4) + 46.4, in which T is the average daily T (°C) and RH is the average relative humidity

digestibility are presented in Table 3. Crude protein intake was reduced in the SIL diet compared to CON (118 vs. 177 g/day, $P<0.001$) in connection with reduced CP content of the SIL diet. DM and CP digestibility were also lower in SIL diet compared to CON (0.81 vs. 0.90 and 0.50 vs. 0.68, respectively, $P<0.001$). Faecal excretion of N was higher in SIL compared to CON (49.2 vs. 38.8 g/day, $P<0.01$), whereas it was the reverse for urinary N excretion, which was lower in SIL compared to CON (8.29 vs. 17.6 g/day, $P<0.001$). There was a significant effect of the diet x temperature interaction only for urinary N excretion ($P<0.05$). In the CON diet, lower urinary N excretion was observed in HS compared to TN, whereas it was the reverse for the animals fed the SIL diet. There was no effect of temperature on nitrogen metabolism or digestibility coefficients.

3.3 Growth performance and carcass parameters

Effect of diet and temperature on growth performance parameters are presented in Table 4. There was no significant difference among groups in Average Daily Gain (ADG) ($P=0.84$). Irrespective of temperature, pigs fed the SIL diet had lower Average Daily Feed Intake (ADFI) than pigs fed the CON diet (710 g/d vs. 862 g/d, $P<0.001$), resulting in improved Feed Efficiency (FE) for the SIL diet (0.34 vs. 0.28, $P<0.05$). No interaction between temperature and treatment was found for any growth performance trait ($P>0.05$).

The slaughter performance reflected the growth performance (Table 4), with no effect of temperature or diet on slaughter BW nor hot carcass weight. SIL diet reduced perirenal fat (21.7 g vs. 35.8 g, $P<0.001$), liver (769.6 g vs. 945.0 g, $P<0.001$) and total viscera weight (1578 g vs. 1790 g, $P<0.01$) compared to CON diet. The only organ size affected by temperature was kidney, with reduced kidney weight in HS group compared to TN (148 g vs. 169 g, $P<0.05$). No interaction between temperature and treatment was found for any slaughter performance trait ($P>0.05$).

Table 3 Effect of temperature and diet on digestibility and nitrogen metabolism

Item	TN-CON	TN-SIL	HS-CON	HS-SIL	RSD[1]	Significant effect[2]
Number of pigs	12	12	12	12		
Intake (g/day)						
DM	965	812	928	787	66.9	R***, D***, D x W***
Digestibility						
DM	0.90	0.80	0.90	0.82	0.06	D***, W**, T x W**
OM	0.91	0.82	0.91	0.83	0.06	D***, W**, T x W**
CP	0.68	0.48	0.68	0.52	0.15	D***, T x W*
ADF	0.60	0.48	0.58	0.51	0.22	R**, D*, W***
ADL	0.11	0.09	0.10	0.21	0.43	R**, W*
Nitrogen metabolism (g/day)						
Intake	181	116	173	119	10.3	R***, D***, W***, D x W***, T x W***, D x T x W*
Fecal excretion	40.0	51.8	37.6	46.6	20.4	R***, D**, W***, D x W***
Urinary excretion	18.3	7.3	16.9	9.3	4.56	R***, D***, D x T*, D x W*, T x W**
Retention	123	57	118	63	22.4	D***, W***, D x W***, T x W***

[1] Residual Standard Deviation. [2] From an analysis of variance with a linear mixed model including the effects of Diet (D), Temperature (T), Replicate (R), Week (W) and their interactions as fixed effect. Statistical significance: ***$P<0.001$, **$P<0.01$, *$P<0.05$, †$P\leq0.10$. [3] BW = Body Weight. [4] ADFI = Average Daily Feed Intake. [5] ADG = Average Daily Gain. [6] BFT = Backfat Thickness. [7] FE = Feed Efficiency

Table 4 Effect of temperature and diet on growth and slaughter traits

Item	TN-CON	TN-SIL	HS-CON	HS-SIL	RSD[1]	Significant effect[2]
Number of pigs	12	12	12	12		
Performance parameters						
Live BW[3], kg						
d0	44.6	42.9	44.7	42.4	1.98	–
d28	50.3	48.0	49.8	47.9	1.98	–
ADFI[4], g DM/d	882	718	842	702	23.5	R***, D***
ADG[6], g/d	233	209	210	228	20.5	R*
FE[9], kg of gain/kg of feed	0.29	0.33	0.27	0.35	0.03	R***, D*
Slaughter parameters						
Slaughter BW, kg	47.0	44.9	47.4	46.2	2.05	–
Hot carcass, kg	36.2	35.3	36.3	36.0	1.67	–
Carcass dressing, %	77.1	79.1	76.5	77.7	1.33	R**
Total viscera, g	1799	1651	1783	1506	73.6	R***, D***
Kidneys	170	168	163	133	11.4	R**, T*, D†
Liver	926	797	964	743	49.0	R***, D***
Perirenal fat	38.3	20.0	33.3	23.3	3.67	D***
Heart	207	202	196	189	14.1	R**
Others	458	465	427	418	27.8	R***, T†

[1] Residual Standard Deviation. [2] From an analysis of variance with a linear mixed model including the effects of Diet (D), Temperature (T), Replicate (R) and their interactions as fixed effect. Statistical significance: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P \leq 0.10$. [3] BW = Body Weight. [4] ADFI = Average Daily Feed Intake. [5] ADG = Average Daily Gain. [7] FE = Feed Efficiency

Table 5 Effect of temperature and diet on thermoregulatory responses

Item	TN-CON	TN-SIL	HS-CON	HS-SIL	RSD[1]	Significant effect[2]
Number of pigs	12	12	12	12		
Rectal Temperature, °C	38.2	37.9	38.2	38.1	0.49	W*, D†, TxW†
Skin Temperature, °C	31.9	31.1	34.5	33.9	1.23	R***, D***, T***, W***, TxW***, TxDxW***
Muscle temperature, °C	37.7	37.2	37.7	37.2	0.53	R*, D**, H***, W***, HxW***, HxT***, HxD***, HxDxT***
Respiratory Rate, bpm	24.6	24.6	31.7	33.0	0.75	R***, T***, H***, W***, TxW***

[1] Residual Standard Deviation. [2] From an analysis of variance with a linear mixed model including the effects of Diet (D), Temperature (T), Week (W) and (H) of measurement, Replicate (R) and their interactions as fixed effect. Statistical significance: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P \leq 0.10$

3.4 Thermoregulatory responses

Table 5 shows the effects of diet and temperature on thermoregulatory responses. There was an effect of temperature on ST and RR, which were increased in HS compared to TN (34.1 °C vs. 31.5 °C and 32.3 bpm vs. 24.6 bpm, $P < 0.001$ respectively). Irrespective of temperature, the SIL diet tended to reduce RT (38.1 °C vs. 37.9 °C, $P = 0.06$) and reduced ST (32.5 °C vs. 33.2 °C, $P < 0.001$) and muscle temperature (37.2 °C vs. 37.8 °C, $P < 0.01$). Respiratory rate was not affected by diet ($P = 0.57$). No interaction between temperature and treatment was found for any thermoregulatory trait ($P > 0.05$).

Continuous measures of muscle temperature at 15-min intervals showed that body temperature followed a similar circadian rhythm in both TN and HS conditions, with lower Tmuscle in SIL compared to CON (Fig. 2). In both environments, Tmuscle peaked once a day at midday (between 1000 h and 1600 h) and reached a minimum at 0500 h. The highest Tmuscle was obtained in the HS environment, at 15:00 (Fig. 2) and corresponds to the peak of ambient T in HS (Fig. 1). Muscle temperature was higher in HS than TN during the day, from 12:00 to 20:00 but lower during the night, from 1:00 to

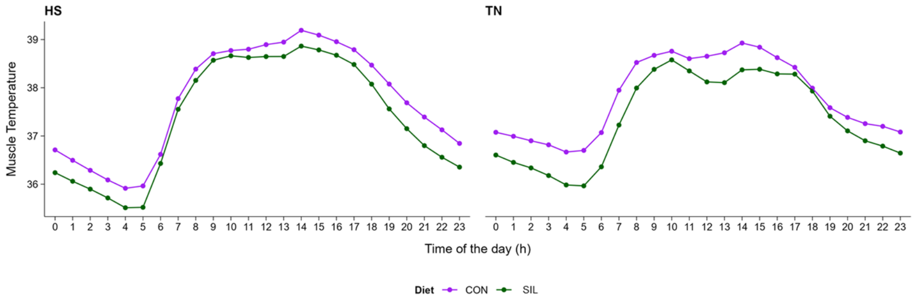

Fig. 2 Hourly variation of muscle temperature according to diet: Control diet (CON, purple) or Diet with sweet potatoes' leaf silage supplemented with free amino acids (SIL, green), and environmental conditions: Thermoneutral (TN, right panel) or Heat Stress (HS, left panel)

Table 6 Effect of temperature and diet on blood biochemical parameters

Item	TN-CON	TN-SIL	HS-CON	HS-SIL	RSD[1]	Significant effect[2]
Number of pigs	12	12	12	12		
ALK Phos, U/L	28.4	28.7	33.7	35.2	0.85	R***, T*, TxW**
GGT, U/L	41.4	40.0	40.7	36.0	1.02	R†, W**, TxW**
AST, U/L	26.3	35.5	27.5	40.3	1.10	W**
ALT, U/L	59.3	84.5	59.3	88.4	1.05	D***, W***
Amylase, U/L	553	546	625	557	1.22	R†, W†, DxW**
BUN, g/L	0.3	0.2	0.3	0.2	0.12	R***, D***, W***, DxTxW*
Glucose, g/L	0.9	0.8	0.8	0.8	0.13	W*, DxT†
Phosphorus, mg/L	89.3	70.5	92.9	74.6	1.01	R†, D**
Albumin, g/L	44.5	41.3	41.2	39.2	1.03	R†, D*, T*
Total protein, g/L	78.6	75.5	77.1	70.9	1.00	R***, D**, T*, TxW*
Globulines, g/L	32.1	33.2	37.7	30.5	4.8	R***, DxT*

[1] Residual Standard Deviation. [2] From an analysis of variance with a linear mixed model including the effects of Diet (D), Temperature (T), Week of measurement (W), Replicate (R) and their interactions as fixed effect. Statistical significance: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P \leq 0.10$. [3] Alkaline Phosphatase. [4] Gamma γ-glutamyl transferase. [5] Aspartate Transaminase. [6] Alanine Transaminase. [7] Blood Urea Nitrogen.

8:00. Muscle temperature in the CON and SIL diet followed a similar pattern throughout the day, with overall lower temperatures for the SIL group.

3.5 Serum biochemical parameters

Effects of diet and temperature are presented in Table 6. Diet impacted different biochemical parameters: BUN (0.16 vs. 0.30 g/L, $P < 0.001$), phosphorus (72.6 vs. 91.1 mg/L, $P < 0.01$), albumin (40.2 vs. 42.9 g/L, $P < 0.05$) and total proteins (73.2 vs. 77.9 g/L, $P < 0.01$) were reduced in SIL compared to CON, whereas alanine transaminase (ALT) increased (86.5 vs. 59.3 U/L, $P < 0.001$). Irrespective of diet, albumin (40.2 vs. 42.9 g/L, $P < 0.05$) and total proteins (74.0 vs. 77.1 g/L, $P < 0.05$) were reduced in HS compared to TN, whereas alkaline phosphatase increased in HS compared to TN (34.4 vs. 28.5 U/L, $P < 0.05$). Globulin level was the only parameter showing a Diet x Temperature interaction effect ($P < 0.05$). In the CON group, levels of globulins increased in HS compared to TN, whereas in SIL group levels were similar in HS and TN (Table 6).

4 Discussion

In the tropics, developing the use of tropical foliage as a protein source for pig feeding could reduce the environmental and economic impact of importing SBM in low input or agroecological mixed farming systems. However, little information is available on the effect of such a diet on growth and metabolism and how it may impact the animal response to the chronic heat stress that the pigs have to face in tropical regions. In the present study, we investigated the effect of replacing part of the protein source of the diet by SP silage on pig performance, thermoregulation and metabolism.

Chemical analysis of the CP content of SP silage show that it was lower than reported in previous studies (110 vs. 187 to 298 g/kg MS [5]). This discrepancy could be due to differences in the plant variety, agricultural practices, such as age at harvesting [6] or the inclusion of variable proportion of stem in the silage whose composition differs from the composition of leaves [16]. Ensiling could also reduce protein content, due to proteolysis occurring after harvesting, during wilting and in the silage itself [5], especially when wilting occurs in humid conditions [23], which was the case in our study. Overall, ADG remained low in both SIL and CON groups (< 250 g/day), probably due to the bulkiness of green banana and its tendency to depress the digestibility of the nitrogen of the ration [24]. The ADG were in concordance with the values found with outdoor pigs pasturing in sweet potatoes fields [25] or in traditional low input systems [26].

The reduction of DFI observed when feeding the SIL diet is likely due to the increased fiber content of the SIL diet, which may have resulted in longer time spent eating [27] or earlier satiety [28]. Similar findings were obtained when feeding pigs with low vs. high fibre diets [29]. The LW breed used in this study could also explain the reduction in feed intake, as exotic breeds with high growth potential and high requirements may have a lower ability to thrive on forage and fiber-rich diet than local breeds [5, 30].

Faecal nitrogen excretion was higher in SIL diet than CON, resulting in lower nitrogen retention, which is consistent with results found by Regnier et al. (2013) [16] when testing different tropical foliage in pig diets. Dry matter digestibility was lower for the SIL compared to the CON diet but values were higher than those obtained in studies using dried SP foliage [7, 16], suggesting that ensiling resulted in higher digestibility than drying. This hypothesis is in accordance with other studies comparing dried and ensiled cassava leaves [7].

Despite reduced feed intake and lower nitrogen retention, the SIL diet allowed similar growth than the CON diet, resulting in better FE for the SIL diet. This could be explained by the combination of two factors. First, the supplementation with free AA of the SIL diet may have compensated its low CP content. Similar growth between pigs fed high- or reduced-CP AA-supplemented diets has been shown previously [28, 31, 32]. Similarly, studies in which a cassava leaves diet was supplemented with DL-methionine and L-Lysine showed increased ADG in the AA supplemented diet compared to the basal diet [12]. The second factor explaining similar ADG despite reduced ADFI and lower nitrogen content, is that the partitioning of lipid and protein could be altered in the SIL diet, similarly to what happens during feed restriction. Two opposite mechanisms may be at play, on one hand, CP availability is reduced and a greater part of metabolizable energy is used for maintenance [33, 34] but on the other hand, a greater proportion of retained energy is accumulated as protein, rather than lipid [33, 35]. Due to the association of water with protein, the reduction of ADG may be lower than the reduction of DFI. A reduction of feed intake is also accompanied by a reduction of heat production, resting metabolic rate [33] and an increase in whole-body fat oxidation to maintain homeostasis [36]. This is consistent with our findings of reduced thermoregulatory measures (ST, RR and Tmuscle) and reduced perirenal fat in pigs fed the SIL diet. Visceral organ weight, which is energetically expensive to maintain and directly related to fasting heat production and feed intake [29, 37], was also reduced in pigs fed the SIL diet. In line with our observations of reduced thermoregulatory parameters in the SIL diet, previous studies in pigs showed that reducing CP in the diet reduces heat production, while the addition of crystalline AA improves AA absorption and minimizes digestive heat production because AA can be absorbed faster than protein-bound AA [38, 39].

Blood biochemical analysis confirmed that protein metabolism was affected by the diet. Blood urea nitrogen, which has been demonstrated to be a good indicator to assess adequate CP content in the diet of growing and finishing pigs [40], was reduced in the SIL diet, confirming the low CP content of the diet. Total protein and albumin were also reduced in the SIL diet compared to the CON diet. Albumin is the major plasma protein, and a reduction in albumin may indicate a reduction in protein synthesis, suggesting that AA-supplementation and fat tissue mobilization in the SIL diet is not sufficient to maintain protein synthesis. Reduction of plasma protein and albumin concentrations were observed in restrictedly-fed chickens compared to their ad-libitum counterparts [41]. Such a reduction of blood total protein and albumin could impact pig immune response and health. Tang et al. [42] showed that reducing dietary protein levels impacted the titer of Swine Fever Virus antibodies and mRNA levels of cytokines in the serum of growing pigs. Further studies measuring immunoglobulins and cytokines in pigs fed with the SIL diet would be necessary to conclude on potential health consequences.

We also found that levels of ALT increased in the SIL diet compared to CON. Alanine transaminase is an intermediary enzyme that plays a pivotal role in glucose and protein metabolism. Alanine transaminase is particularly important for energy homeostasis during fasting and prolonged exercise when muscle proteins must first be broken down into its constituent amino acids [43]. Therefore, this result could suggest a mobilization of muscle proteins in the SIL diet to compensate for the reduced CP levels of the SIL diet.

No significant effect of temperature or temperature x diet interaction was observed on nitrogen metabolism or performance traits. This is probably due to the reduced temperature gap between TN and HS environment. Despite having higher temperature during the day, the HS environment had lower temperature at night, which may have allowed the animals to compensate for the HS of the day. Consistent with this hypothesis, muscle T was higher in HS compared to TN during the day but was lower at night. Skin temperature and RR, which were measured during the day, were consistently higher in HS compared to TN. Moreover, in a previous study using the same animals but focusing on behaviour, we found that the HS condition induced changes in pig behaviour, with pigs adopting the lateral lying position to dissipate heat more efficiently [44]. As reviewed in Vermeer and Aarninck [20], pigs as homeotherms, have different thermoregulation strategies depending on the ambient temperature. In our HS conditions, during daytime, THI is above the pigs' comfort zone (THI > 79–80 for 30–40 kg pigs [20]), which explains the change in behaviour and thermoregulatory response, but remains under Upper Critical Temperature (UCT, THI > 85 for 30–40 kg pigs [20]) at which pigs start to reduce their FI to lower heat generated by feeding and digestion.

Regarding the effect of HS on biochemical blood parameters, total protein and albumin were significantly reduced in HS, probably linked to the numerical reduction of ADFI and CP intake in HS compared to TN. Moreover, HS affects the intestinal barrier and reduces AA digestibility [10], which could explain the reduction in total protein and albumin levels observed. A meta-analysis on the effects of cyclic and constant HS showed that serum albumin was reduced in pigs submitted to constant HS and that it may be related to the reduction in lipid deposition during HS [45]. We found that globulin increased in HS compared to TN in the CON diet. This is consistent with previous finding in pigs subjected to HS and could suggest immune activation due to chronic inflammation [46]. Interestingly, this increase of circulating globulin was not observed in SIL diet, when comparing TN and HS. The concentration of AA in the diet may modify the immune response of the pigs [47, 48], which could explain this result. However, further studies on the type of globulin involved should be carried out to validate this hypothesis.

In conclusion, our results demonstrate that replacing 70% of CP with SP silage reduces DFI and increases nitrogen excretion. Biochemical blood parameters confirmed that protein metabolism was affected by the diet. However, performance was maintained at similar levels in both diets, probably through the supplementation with free essential AA in the SIL diet, but also because of changes in the partition of protein and lipids in response to feed restriction. The effect of diet on the animal response to heat could not be fully examined due to heat stress being moderate during our experimental conditions. Nevertheless, thermoregulatory measures were reduced in the SIL diet, irrespective of temperature, suggesting lower heat production when animal are fed with this diet. Further experiments at higher temperatures and including local breeds should be carried out to investigate further the effect of feeding tropical foliage diet on pig heat stress response.

Regarding practical applications, our study demonstrates that replacing SBM by SP silage in the ration of growing pigs could be an economical and environmental-relevant alternative for low input or agroecological mixed farming systems in the tropics. However, protein levels in SP silage can be variable and supplementation with free essential AA may be key to maintain growth performance.

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Author contributions

NP, JLG and JCB conceived and designed the experiment. NP and JG collected the data. DB and LD were in charge of the experiments at the animal experimental unit. DF, YF and JG performed the laboratory analysis on blood samples. JLG, JCB, and NP discussed and interpreted the results. NP and JG analysed the data and wrote the original draft. JLG and JCB reviewed and edited the paper. All authors read and approved the final manuscript.

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Data availability

The data used to support the findings of this study are included in the article.

Declarations

Ethics approval and consent to participate

All measurements and observations on animals were performed in accordance with relevant guidelines and regulations on animal experimentation and ethics. All experimental protocols were approved by the Animal Care and Use Committee of French West Indies and Guyana (#69-2012-2) and the French Ministry of Agriculture authorized the study under the reference APAFIS#6070-2016070721289156v3 on living animals at the INRAE facility under the direction of N. Minatchy (INRAE-PTEA). All plant material used in this study was collected from cultivated plants, following relevant national guidelines on plant collection.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Hermansen JE, Kristensen T. Management options to reduce the carbon footprint of livestock products. Anim Front. 2011;1(1):33–9. https://doi.org/10.2527/af.2011-0008.
2. da Prudêncio Silva V, van der Werf HMG, Spies A, Soares SR. Variability in environmental impacts of Brazilian soybean according to crop production and transport scenarios. J Environ Manage. 2010;91(9):1831–9. https://doi.org/10.1016/j.jenvman.2010.04.001.
3. FAOSTAT. https://www.fao.org/faostat/en/#data/QCL. Accessed 17 Oct 2025.
4. Stark F, Fanchone A, Semjen I, Moulin C-H, Archimède H. Crop-livestock integration, from single practice to global functioning in the tropics: case studies in Guadeloupe. Eur J Agron. 2016;80:9–20. https://doi.org/10.1016/j.eja.2016.06.004.
5. Kambashi B, Boudry C, Picron P, Bindelle J. Forage plants as an alternative feed resource for sustainable pig production in the tropics: a review. Animal. 2014;8(8):1298–311. https://doi.org/10.1017/S1751731114000561.
6. Van An L, Lindberg JE. Ensiling of sweet potato leaves (*Ipomoea batatas* (L.) Lam) and the nutritive value of sweet potato leaf silage for growing pigs. Asian-Australas J Anim Sci. 2004;17(4):497–503. https://doi.org/10.5713/ajas.2004.497.
7. Nguyen THL, Ngoan LD, Bosch G, Verstegen MWA, Hendriks WH. Ileal and total tract apparent crude protein and amino acid digestibility of ensiled and dried cassava leaves and sweet potato vines in growing pigs. Anim Feed Sci Technol. 2012;172(3):171–9. https://doi.org/10.1016/j.anifeedsci.2011.11.009.
8. Régnier C, Jaguelin Y, Noblet J, Renaudeau D. Ileal digestibility of amino acids of cassava, sweet potato, cocoyam and erythrina foliages fed to growing pigs. animal. 2012;6(4):586–93. https://doi.org/10.1017/S1751731111001959.
9. Pearce SC, et al. Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. PLoS One. 2013;8(8):e70215. https://doi.org/10.1371/journal.pone.0070215.
10. Morales A, et al. Effect of heat stress on the endogenous intestinal loss of amino acids in growing pigs. J Anim Sci. 2016;94(1):165–72. https://doi.org/10.2527/jas.2015-9393.
11. Morales A, et al. Effect of dl-methionine supplementation above requirement on performance; intestinal morphology, antioxidant activity, and gene expression; and serum concentration of amino acids in heat stressed pigs. J Anim Sci. 2023;101:skac379. https://doi.org/10.1093/jas/skac379.
12. Ly NTH, Ngoan LD, Verstegen MWA, Hendriks WH. Pig performance increases with the addition of dl-methionine and l-lysine to ensiled cassava leaf protein diets. Trop Anim Health Prod. 2012;44(1):165–72. https://doi.org/10.1007/s11250-011-9904-3.
13. Noblet J et, Milgen JV. Energy value of pig feeds: effect of pig body weight and energy evaluation system. J Anim Sci. 2004;82:229–38. https://doi.org/10.2527/2004.8213_supplE229x.
14. Gourdine J-L et al. Systèmes de production valorisant des ressources locales en production porcine en milieu tropical. 2011.
15. Renaudeau D, et al. Banana meal for feeding pigs: digestive utilization, growth performance and feeding behavior. Animal. 2014;8(4):565–71. https://doi.org/10.1017/S175173111400010X.
16. Regnier C, Bocage B, Archimede H, Noblet J, Renaudeau D. Digestive utilization of tropical foliages of cassava, sweet potatoes, wild cocoyam and erythrina in Creole growing pigs. Anim Feed Sci Technol. 2013;180(1-4):44–54. https://doi.org/10.1016/j.anifeedsci.2012.12.007.
17. Renaudeau D. Evaluation of a telemetry system for measuring core body temperature in the pig. J Rech Porc En Fr. 2016;48:249–50.
18. Official Methods of Analysis, 22nd Edition. AOAC INTERNATIONAL. Consulté le: 17 octobre 2025. [En ligne]. 2023. Disponible sur: https://www.aoac.org/official-methods-of-analysis/
19. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991;74(10):3583–97. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
20. Vermeer HM, Aarnink AJA. Review on heat stress in pigs on farm. Eur Union Ref Cent Anim Welf Pigs. 2023. https://doi.org/10.5281/zenodo.7620726.
21. Pinheiro J, Bates D, R Core Team. nlme: Linear and nonlinear mixed effects models, version 3.1-168. R Core Team. 1999. https://doi.org/10.32614/CRAN.package.nlme.
22. Lenth RV et, Piaskowski J. emmeans: Estimated Marginal Means, aka Least-Squares Means. [En ligne]. 2025. Disponible sur: https://rvlenth.github.io/emmeans/
23. Webster J. The Biochemistry of Silage (Second Edition). By P. McDonald, A. R. Henderson and S. J. E. Heron. Marlow, Bucks, UK: Chalcombe Publications, (1991), pp. 340, £49.50, ISBN 0-948617-225. Exp Agric. 1992;28(1):125–125. https://doi.org/10.1017/S0014479700023115.
24. Le Dividich J, Geoffroy F, Canope I, Chenost M. Utilisation des déchets de banane pour l'alimentation du bétail. Rev Mond Zootech. 1976;20:22.
25. Gourdine J-L, Bambou JC, Giorgi M, Loranger-Merciris G, Archimede H. Performance of growing pigs reared indoors or outdoors in sweet-potato fields. Rev Elev Med Vet Pays Trop. 2018;71(1–2):41–6.
26. Kumaresan A, et al. Performance of pigs reared under traditional tribal low input production system and chemical composition of non-conventional tropical plants used as pig feed. Livest Sci. 2007;107(2–3):294–8. https://doi.org/10.1016/j.livsci.2006.12.007.
27. Whittemore EC, Kyriazakis I, Tolkamp BJ, Emmans GC. The short-term feeding behavior of growing pigs fed foods differing in bulk content. Physiol Behav. 2002;76(1):131–41. https://doi.org/10.1016/S0031-9384(02)00686-8.

28. Kerr BJ, Southern LL, Bidner TD, Friesen KG, Easter RA. Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. J Anim Sci. 2003; 81(12):3075–3087. https://doi.org/10.2527/2003.81123075x
29. Gondret F, et al. Dietary energy sources affect the partition of body lipids and the hierarchy of energy metabolic pathways in growing pigs differing in feed efficiency. J Anim Sci. 2014;92(11):4865–77. https://doi.org/10.2527/jas.2014-7995.
30. Len NT, Hong TT, Ogle B, Lindberg JE. Comparison of total tract digestibility, development of visceral organs and digestive tract of Mong cai and Yorkshire× Landrace piglets fed diets with different fibre sources. J Anim Physiol Anim Nutr. 2009;93(2):181–91. https://doi.org/10.1111/j.1439-0396.2007.00804.x.
31. Kerr BJ, Easter RA. Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. J Anim Sci. 1995;73(10):3000–8. https://doi.org/10.2527/1995.73103000x.
32. Knowles TA, Southern LL, Bidner TD, Kerr BJ, Friesen KG. Effect of dietary fiber or fat in low-crude protein, crystalline amino acid-supplemented diets for finishing pigs. J Anim Sci. 1998;76(11):2818–32. https://doi.org/10.2527/1998.76112818x.
33. Lovatto PA, Sauvant D, Noblet J, Dubois S, Milgen JV. Effects of feed restriction and subsequent refeeding on energy utilization in growing pigs. J Anim Sci. 2006;84:3329–36. https://doi.org/10.2527/jas.2006-048.
34. Hornick JL, Eenaeme CV, Gérard O, Dufrasne I, Istasse L. Mechanisms of reduced and compensatory growth. Domest Anim Endocrinol. 2000;19(2):121–32. https://doi.org/10.1016/S0739-7240(00)00072-2.
35. De Greef KH, Kemp B, Verstegen MW. Performance and body composition of fattening pigs of two strains during protein deficiency and subsequent realimentation. Livest Prod Sci. 1992;30:141–53.
36. Chwalibog A, Tauson A-H, Thorbek G. Energy metabolism and substrate oxidation in pigs during feeding, starvation and re-feeding. J Anim Physiol Anim Nutr. 2004;88(3-4):101–12. https://doi.org/10.1111/j.1439-0396.2003.00465.x.
37. Koong L-J, Nienaber JA, Pekas JC, Yen J-T. Effects of plane of nutrition on organ size and fasting heat production in pigs. J Nutr. 1982;112(8):1638–42. https://doi.org/10.1093/jn/112.8.1638.
38. Morales A, et al. Effect of feed intake level and dietary protein content on the body temperature of pigs housed under thermo neutral conditions. J Anim Physiol Anim Nutr. 2018;102(2):e718–25. https://doi.org/10.1111/jpn.12824.
39. Morales A, et al. Increased dietary protein or free amino acids supply for heat stress pigs: effect on performance and carcass traits. J Anim Sci. 2018;96(4):1419–29. https://doi.org/10.1093/jas/sky044.
40. Camp Montoro J, Solà-Oriol D, Muns R, Gasa J, Llanes N, Manzanilla EG. Blood and faecal biomarkers to assess dietary energy, protein and amino acid efficiency of utilization by growing and finishing pigs. Porc Health Manag. 2022;8(1):32. https://doi.org/10.1186/s40813-022-00273-y.
41. Rajman M, et al. The effects of feed restriction on plasma biochemistry in growing meat type chickens (*Gallus gallus*). Compar Biochem Physiol Part A Mol Integr Physiol. 2006;145(3):363–71. https://doi.org/10.1016/J.CBPA.2006.07.004.
42. Tang Y, et al. The effect of dietary protein intake on immune status in pigs of different genotypes. Food Agric Immunol. 2025. https://doi.org/10.1080/09540105.2018.1455812.
43. Qian K, Zhong S, Xie K, Yu D, Yang R, Gong D-W. Hepatic ALT isoenzymes are elevated in gluconeogenic conditions including diabetes and suppressed by insulin at the protein level. Diabetes Metab Res Rev. 2015;31(6):562–71. https://doi.org/10.1002/dmrr.2655.
44. Poullet N, Guichard J, Beramice D, Dantec L, Gourdine J-L, Bonneau M. Combining automated behaviour recognition and physiological data to characterize heat tolerance and animal welfare in growing pigs. J Therm Biol. 2025. https://doi.org/10.1016/j.jtherbio.2025.104048.
45. de Oliveira MJK, et al. Effect of constant and cyclic heat stress on growth performance, water intake, and physiological responses in pigs: a meta-analysis. Anim Feed Sci Technol. 2024;309:115904. https://doi.org/10.1016/j.anifeedsci.2024.115904.
46. Rudolph TE, et al. Heat stress alters hematological parameters in barrows and gilts. J Anim Sci. 2024;102:skae123. https://doi.org/10.1093/jas/skae123.
47. de José Karpeggiane Oliveira M, et al. Effects of lowering dietary protein content without or with increased protein-bound and feed-grade amino acids supply on growth performance, body composition, metabolism, and acute-phase protein of finishing pigs under daily cyclic heat stress. J Anim Sci. 2022;101:skac387. https://doi.org/10.1093/jas/skac387.
48. Prates JAM, et al. Influence of dietary supplementation with an amino acid mixture on inflammatory markers, immune status and serum proteome in LPS-challenged weaned piglets. Animals. 2021;11(4):1143. https://doi.org/10.3390/ani11041143.

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