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Original article

The incorporation of solubilized wheat proteins in milk replacers for veal calves: effects on growth performance and muscle oxidative capacity*

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Abstract — Replacement of skim milk proteins by solubilized wheat protein (SWP) in milk replacers for veal calves would contribute to the reduction in feeding costs. The occurrence of metabolic disorders has, however, been reported. Forty-two male calves received one of three treatments over 140 days: a control diet, a diet containing SWP without or with branched-chain amino acid supplementation. Liveweight gain, carcass yield, color and conformation did not show any significant differences. No metabolic disorders were noted. Supplementation with branched-chain amino acids reduced the marginal Val deficiency but did not modify the growth performances. With the SWP containing diets, the plasma metabolite profile was characteristic of those observed with non-clotting diets. It was statistically correlated to the changes in the orientation of the *Semitendinosus* muscle energy metabolism towards a more oxidative type and to indications of a lower efficiency of amino acid utilisation for protein deposition. At the present levels of inclusion, SWP proved to be an interesting alternative to the sole use of whey as the protein source in milk replacers for veal calves.

veal / preruminant calf / wheat proteins / milk replacer / growth / blood parameters

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1. INTRODUCTION

In veal calf production systems, the reduction of feeding costs remains of paramount importance. In this purpose, attempts have been made to replace skim milk proteins by proteins of plant origin in milk replacers. The shift from skim milk proteins to protein substitutes has created situations in which abomasal curd formation does not occur [1]. Clotting has long been considered to be essential for adequate nutrient digestion and absorption in calves, and subsequently for satisfactory growth performances. For example, recent data in human beings has demonstrated that the speed of absorption of dietary protein could affect the postprandial protein synthesis, breakdown and deposition [2]. However, other data challenge this conclusion and suggest that the nature of the protein source, its processing method and its nutritional quality are more important to calf performance than its clotting ability [3]. Solubilized wheat proteins (SWP), in particular, are interesting because they are relatively cheap, fairly functional, devoid of anti-nutritional activities and highly digestible [1]. These proteins, are, however, deficient in essential amino acids, including lysine (Lys) and threonine (Thr) as well as branch-chain amino acids (BCAA), i.e. Valine (Val), Leucine (Leu), and Isoleucine (Ile) [1]. In experimental conditions, total replacement of skim milk powder by SWP (providing 65% of the total crude protein) and whey powder results in a 5% decrease of live weight gain and feed efficiency [4] which could result from an amino acid imbalance and/or from metabolic disorders (such as acidosis, high plasma lactate and ammonia concentrations).

Consequently, the objectives of the present experiment were to test in veal calves the effects of including high levels of SWP (supplemented with Lys and Thr) in the place of milk proteins, with or without a supplementation with BCAA on (1) growth performance and the general health status of animals in controlled conditions, (2) the kinetics of the appearance of the metabolic disorders as assessed by the evolution of blood profiles with time, and (3) the orientation of muscle energy metabolism.

2. MATERIALS AND METHODS

2.1. Animals

A total of forty-two 8 to 10 day old Prim'Holstein male calves of an average initial weight of 45 to 50 kg were used. Overall the experiment lasted 146 days which included an adaptation period to the general facilities (d 0 to d 28) followed by a growing (d 29 to d 83) and a finishing (d 84 to d 146) phase. At d 29, the calves were blocked by weight and the hematocrit level, and were randomly assigned, within a block, to one of the three treatment groups: Control (C), SWP or SWP+BCAA. The animals were housed in conventional building conditions for veal calves, with the ventilation varying between 50 and 250 m^3/h/animal depending on the temperature in the building. The blocks were randomly assigned to one of two experimental rooms. One of the rooms housed 6 blocks of 3 animals each in individual cages (1.5 m^2/calf), while the other room housed 8 blocks i.e. 24 calves in collective cages by groups of two (1.5 m^2/calf). The experiment was conducted in a manner compatible with the national legislation on animal care (Certificate of Authorization to Experiments on Living Animals, nº 004495, Ministry of Agriculture).

2.2. Diets and allowances

The SWP concentrate was a commercial product prepared by partial enzymatic hydrolysis. It contained 842 g crude protein (CP) per kg dry matter. Three milk substitute diets (Tabs. I and II) were prepared and

Table I. Composition of the diets of the growing phase (g·kg^{-1} powder).

	Diets		
	Control	SWP	SWP+BCAA
INGREDIENT COMPOSITION			
– tallow + whey powder premix[a]	286	280	280
– lard + whey powder premix[a]	90	88	88
– coconut oil + skim milk powder premix[a]	90	–	–
– coconut oil + whey powder premix[a]	–	88	88
– skim milk powder	448	–	
– whey powder	–	148	148
– whey protein concentrate (ultrafiltration)	45	78	78
– whey protein concentrate (nanofiltration)	–	98	98
– soluble wheat protein concentrate[b]	–	149	149
– wheat flour	30	29	29
– L-Lysine-HCl	0.80	8.04	8.04
– Dl-methionine	0.60	0.59	0.59
– threonine	–	1.37	1.37
– valine	–	–	2.84
– isoleucine	–	–	0.49
– leucine	–	–	1.96
– dicalcium phosphate	–	2.9	2.9
– calcium carbonate	–	6.9	6.9
– lactose	–	11.0	5.7
– minerals, vitamins and antibiotics[c]	9.6	9.4	9.4
CHEMICAL COMPOSITION (calculated)			
– crude protein	211	228	231
– fat	188	192	192
– minerals	69	68	68
– Ca	8	8	8
– P	7	5	5
– Mg	1.3	1.3	1.3
– K	14	13	13
– Na	5	6	6
– Cl	12	10	10

[a] Mixture of fat and concentrated whey homogenized and then spray-dried (≈ 400 g fat·kg^{-1} powder).
[b] Solpro 500 (Amylum N.V., Aalst, Belgium).
[c] EUROVO (Bourgbarré, France).

Table II. Composition of the diets of the finishing phase ($g \cdot kg^{-1}$ powder).

	Diets		
	Control	SWP	SWP+BCAA
INGREDIENT COMPOSITION			
– tallow + whey powder premix[a]	320	314	314
– lard + whey powder premix[a]	130	157	157
– lard + delactosed whey powder premix[a]	20	–	–
– coconut oil + skim milk powder premix[a]	50	–	–
– coconut oil + whey powder premix[a]	–	49	49
– skim milk powder	370	–	–
– whey powder	–	237	237
– whey protein concentrate (ultrafiltration)	65	–	–
– soluble wheat protein concentrate[b]	–	167	167
– wheat flour	30	29	29
– L-Lysine-HCl	1.20	10.20	10.20
– Dl-methionine	1.20	0.59	0.59
– threonine	–	2.75	2.75
– valine	–	–	3.04
– isoleucine	–	–	1.27
– leucine	–	–	2.65
– tryptophan	–	0.30	0.30
– dicalcium phosphate	–	7.8	7.8
– calcium carbonate	–	6.9	6.9
– anti-foaming agent	2	2	2
– lactose	–	7	–
– minerals, vitamins and antibiotics[c]	10.6	9.6	9.6
CHEMICAL COMPOSITION (calculated)			
– crude protein	203	210	215
– fat	210	214	214
– minerals	66	61	61
– Ca	8	8	8
– P	7	5	5
– Mg	1.4	0.9	0.9
– K	15	11	11
– Na	5	5	5
– Cl	12	8	8

[a, b, c] See Table I.

distributed to one of the three treatment groups. Protein for the C diet came almost exclusively from spray-dried skim milk and whey products (83 and 14% from d 29 to d 83, 69 and 28% from d 84 to d 146, respectively). In the SWP and SWP-BCAA diets, the protein from skim milk powder was replaced by the SWP concentrate and whey products. The SWP concentrate provided 49% of the total CP from d 29 to d 83 and 61% from d 84 to d 146. Both SWP and SWP+BCAA diets were supplemented with Lys and Thr (plus Tryptophane (Trp) during the finishing phase), whereas the SWP+BCAA diet was also supplemented with Val, Ile and Leu up to levels similar to those calculated in the C diet.

Feed allowances were set at 95% of ad libitum levels; they allowed to reach a carcass weight close to 130 kg at 140 days. Allowances were adjusted every 3 days. To comply with production conditions, dextran-iron was individually injected intramuscularly according to the degree of anemia when the hematocrit level was lower than 0.32 at d 28 and 0.27 at d 84.

All calves were individually fed, and received two equal meals par day at 7 am and 5 pm.

2.3. Measurements

Individual food intake was measured every day. Live weights and hematocrit levels were determined every 4 wk, at a fixed interval, postprandially. The health status of each animal was recorded daily.

Jugular blood samples were taken on all animals 2.5 h postprandially on days 7, 55, 69, 84, 106, 126 and 138. A total of 15 mL blood was sampled, for half on heparin as the anticoagulant and iniprol as the antipeptidase, and for half on EDTA. Heparinized blood was used immediately for the determination of blood pH, pO_2 and pCO_2. The remainder was centrifuged and the plasma was used (1) after deproteinization with sulphosalicylic acid for the determination of free amino acid levels [1], and (2) after storage at –15 °C for the determination of glucose [5], lactate [6], insulin (Pasteur radioactive immuno-assay n° 79854), cortisol (d 106 only, [7] and tri-iodothyronine (d 106 only, Incstar Corp. CA 1541) concentrations. EDTA plasma was stored frozen for the subsequent determination of urea [8], ammonia [8], β-hydroxybutyrate [9], triglyceride (TG; kit 6.123.8 Biomérieux) and non-esterified fatty acid (NEFA), [10] concentrations.

Muscle biopsy samples of the *Semitendinosus* muscle were taken on all animals on d 111 as previously described [11]. They were immediately frozen in liquid N_2 and stored at –80 °C, awaiting the determination of total muscle protein [12] and DNA [13] contents as well as the activities of oxidative metabolism enzymes (citrate synthase (CS) [14]; and cytochrome-c-oxydase (COX) [15] and glycolytic metabolism enzymes (phosphofructokinase (PFK) [16]; and lactate dehydrogenase (LDH) [17].

The animals were slaughtered on d 146. Carcass characteristics and weight were recorded at slaughter (Tab. III).

2.4. Statistical analysis

All data were analyzed according to a factorial nested design using the GLM procedure of SAS [18]. The main effects considered were housing (individual versus collective cages), treatment and block-housing. The treatment × housing interaction was included in the analysis. The housing effect was tested against the block-housing for the F test. Additional covariates ('hematocrit level on d29' both alone and in interaction with 'treatment') were used for the analysis of the hematocrit levels on d 86 and d 146 and carcass color. In case of significant treatment effects, individual treatment means were compared by the Student t-test. In order to test the kinetics of

Table III. Influence of the substitution of soluble wheat proteins (SWP) for skim milk proteins in milk replacers, with or without branch-chain amino acid supplementation (BCAA), on growth performance and carcass characteristics in veal calves. LSMeans are reported.

	Control	SWP	SWP+BCAA	SEM[+]	Statistics[++]
Live-weight, kg					
d29	60.2	61.6	61.9	0.65	NS
d86	129.5	131.9	132.3	1.42	NS
d146	220.5	220.9	223.6	3.06	NS
Live-weight gain, g·d^{-1}					
Growing phase	1216	1232	1235	22.2	NS
Finishing phase	1516	1484	1522	34.9	NS
Intake, g·d^{-1}					
Growing phase	1675[a]	1718[b]	1722[b]	5.2	T***
Finishing phase	2830	2912	2899	23.9	NS
Feed efficiency, kg feed·kg^{-1} gain					
Growing phase	1.38	1.40	1.40	0.025	NS
Finishing phase	1.88	1.96	1.91	0.035	NS
Hematocrit level,%					
d29	29.5	28.8	28.8	0.96	NS
d86	24.4[a]	26.7[b]	27.4[b]	0.76	T*
d146	24.6	24.8	27.4	0.95	NS
Liver weight, kg	5.24	5.12	4.97	0.139	NS
Cold carcass weight, kg [a]	127.9	128.8	129.5	1.918	NS
Dressing percentage,%	58.0	58.3	57.9	0.36	H*
Carcass color [b]	2.33	2.40	2.32	0.150	H**
Conformation [b]	8.9	9.2	8.8	0.22	NS
Body condition score [b]	8.3	8.2	8.2	0.18	NS

[+] SEM: standard error of treatment means (n = 12).

[++] T: treatment effect, H: housing effect, *P < 0.05, **P < 0.01, ***P < 0.001, NS: not significant.

[a] Carcass weight at slaughter × 0.98.

[b] Carcass characteristics estimated according to the EUROP scale: color from 1 (white) to 4 (red), conformation from 4 to 15, corresponding to P– (insufficient) to U+ (excellent), respectively, body condition score from 2 (insufficient) to 11 (excessive), respectively.

appearance of metabolic disorders within individual treatment groups, the effect of time was tested by the paired t-test within each treatment group.

Principal component analyses were performed using the Factor procedure of SAS [18] to study the relationships between growth parameters, plasma metabolites and

hormones and muscle metabolic characteristics. As for the growth parameters, the following variables were used: total average daily gain (ADG), total feed intake (Intake), total feed efficiency (Feed Eff), cold carcass weight (Carcass), carcass color (Color), conformation (Conf), body condition score (BCS), liver weight (Liver) and dressing percentage (Dressing%). As for the plasma metabolites and hormones, the following variables measured on d 106 were used: plasma concentrations of glucose, insulin, lactate, NEFA, TG, β-hydroxy-butyrate (BOH), ammonia (NH_3), urea, T3 and cortisol, total blood hemoglobin levels (HB), blood pH and pO_2. As far as the muscle metabolic characteristics are concerned, the ratios between the parameters were used since they were judged to be more discriminant. They were the following: ratios between enzymatic activities CS/LDH, COX/LDH, CS/PFK, COX/PFK and the ratio of muscle protein over DNA concentrations (Prot/DNA). The factor axes were calculated from the eigenvectors of the *p* variables and *n* calves of the correlation matrix of the data.

3. RESULTS

3.1. Health and hematocrit status

A total of five animals were eliminated in the course of the experiment. Three calves (one from each treatment) had shown recurrent respiratory and digestive pathologies. Two additional animals from the SWP+BCAA treatment group had presented an ulcer for one and an abscess for the other. For this reason, the LSMeans are reported.

As far as the general health status of the animals was concerned, digestive disorders were not frequent (0.2–0.3 days of administration of medication per animal, on average, in each of the growing and finishing phases) whereas respiratory disorders were important during the growing phase (7.1 days of administration of medication per animal, on average) but not during the finishing phase (0.6 days/animal). These health problems were not significantly influenced by the treatments.

The degree of anemia of veal calves (Tab. III) is an important criterion for veal meat production since white meat is required by the consumer. At the beginning of the experiment, the hematocrit level of all calves was similar (0.29 on average). Subsequently and from d 86 onwards, the hematocrit levels were significantly higher by 2 to 3 percentage units in the SWP and SWP+BCAA calves than in the C calves, despite similar iron supplementation (approximately 250 mg per calf) across the treatment groups. At slaughter, the SWP+BCAA calves still showed a tendency for higher hematocrit levels (0.274) than the C and SWP calves (0.246 and 0.248, respectively; SEM = 0.0095) although this effect was no longer significant. Throughout the finishing phase, the animals in collective housing showed hematocrit levels which were higher by 1 to 4 percentage units ($P < 0.05$) than those of animals in individual housing (results not shown).

3.2. Live weight gain and carcass characteristics

Growth performances were similar for all three treatment groups. Live-weight gains averaged 1228 and 1507 $g \cdot d^{-1}$ in the growing and finishing phases respectively. The introduction of SWP concentrate in the diet, with or without BCAA supplementation, did not significantly modify the live-weight gains, nor feed efficiency (1.39 and 1.92 kg feed intake$\cdot kg^{-1}$ body weight gain on average in the growing and finishing phases, respectively) (Tab. III). Body condition score and carcass characteristics (color, conformation, dressing percentage) were not modified by the inclusion of SWP concentrate with or without BCAA

supplementation in the diet (Tab. III). However, the calves in collective housing had a higher carcass color index ($P < 0.01$) and a lower dressing percentage ($P < 0.05$) than the calves in individual housing (2.61 and 57.4% vs. 2.09 and 58.7%, respectively). The difference in carcass color is likely to be related to the effect of housing on the hematocrit levels, probably due to more spontaneous movements when the calves were housed by groups of two. Liver weights were not affected by the treatment, however they were 7% lower ($P = 0.11$) in collective than in individual housing (4.82 vs. 5.40 kg).

3.3. Plasma amino acid concentrations

Differences in plasma amino acid levels among treatments and with time were determined in order to test whether the applied amino acid supplementations were sufficient to overcome possible amino acid deficiencies with the inclusion of SWP concentrate in the diets. Only the results on essential amino acids are presented here (Tab. IV). For the sake of clarity, only the results obtained at the end of the growing (d 84) and finishing (d 138) phases are reported. When these results were not representative of those obtained during the whole growing or finishing phases, respectively, this is specified in the text.

In the absence of any other changes (gastric emptying rate, balance among amino acids...), plasma amino acid concentrations vary first with the levels of amino acid intake [19], especially the intake in truly digestible amino acids [1]. In the present situation, intake was similar or marginally different between the C and SWP, SWP+BCAA diets for digestible Lys, Thr and Arginine (Arg) (Tab. V). The plasma concentration levels of these three amino acids were, however, significantly higher in SWP and SWP+BCAA calves, probably as a result of a more rapid gastric emptying since blood samples were taken 2.5 h after the last meal. For the SWP calves, this may also be the result of a deficiency in Val. Indeed, when considering the BCAA, plasma Leu and Ile concentrations in SWP calves were never inferior to those measured in the C calves. By contrast, plasma concentrations of Val were significantly lower in the SWP calves as compared to the C calves, in agreement with the differences in intake (Tab. V). On the contrary, the BCAA supplementation restored plasma Val concentrations up to the levels observed in the C calves. These changes point to Val as the major possible limiting amino acid with SWP. Despite a marginal deficiency in the calculated amount of Histidine (His) absorbed from the gut, plasma His concentrations in the SWP and SWP+BCAA calves were always superior to those in the C animals. Plasma concentrations in Methionine (Met) and Phenylalanine (Phe) reflected differences in absorption across the diets.

3.4. Plasma metabolites and hormones

In order to characterize the kinetics of appearance of metabolic disorders in veal calves offered plant proteins, profiles in plasma metabolites and hormones were determined at regular intervals during the growing and finishing phases. Differences were noted among the treatment groups, especially during the growing phase. These differences were essentially attributed to the non-clotting character of the SWP and SWP+BCAA feeds and the calves did not show any specific metabolic disorders. For this reason and to gain in clarity, only the results obtained at the end of the growing (d 84) and finishing (d 138) phases are reported. When these results were not representative of those obtained over the whole growing or finishing phases, respectively, this is specified in the text.

Blood pH varied between 7.37 and 7.41 (Tab. VI). No acidosis and no significant

Table IV. Influence of the substitution of soluble wheat proteins (SWP) for skim milk proteins in milk replacers, with or without branch-chain amino acid supplementation (BCAA), on plasma essential amino acid concentrations measured in calves. LSMeans are reported.

Plasma amino acid concentrations ($mg \cdot L^{-1}$ of plasma)	Control	SWP	SWP+BCAA	SEM [+]	Statistics [++]
Lysine (Lys)					
d84	27.2[a]	38.0[b]	36.1[b]	1.48	T***
d138	30.8[a]	46.6[b]	42.0[b]	3.71	T**
Threonine (Thr)					
d84	17.2[a]	23.5[b]	21.4[b]	0.84	T***, H*
d138	17.5[a]	25.8[b]	22.6[b]	1.11	T***
Valine (Val)					
d84	26.4[a]	21.1[b]	29.2[a]	0.97	T***
d138	28.0[ab]	25.5[a]	33.2[b]	1.45	T*
Leucine (Leu)					
d84	18.9[a]	19.3[a]	22.1[b]	0.79	T*
d138	22.4	23.5	26.4	1.44	NS
Isoleucine (Ile)					
d84	12.9	13.7	13.7	0.52	NS
d138	14.8	15.5	17.0	0.95	NS
Arginine (Arg)					
d84	31.9[a]	47.0[b]	40.5[c]	1.91	T***
d138	26.7[a]	47.6[b]	40.4[b]	2.12	T***
Histidine (His)					
d84	12.4	14.1	13.1	0.67	H*
d138	9.7[a]	15.0[b]	13.0[b]	0.84	T**
Methionine (Met)					
d84	8.1[a]	6.7[b]	6.2[b]	0.37	T*
d138	11.7[a]	8.7[b]	7.5[b]	0.36	T***
Phenylalanine (Phe)					
d84	9.4	10.3	9.4	0.35	H**
d138	9.6[a]	13.2[b]	11.4[c]	0.41	T***

[+] SEM: standard error of treatment means ($n = 12$).
[++] T: treatment effect, H: housing effect, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, NS: not significant.

modifications of the acid-base status of the animals were observed as a result of SWP inclusion in the feeds. Blood pO_2 and pCO_2 results confirmed that the inclusion of SWP concentrate in the feeds did not result in acidosis. On the contrary, blood pCO_2 was significantly lower in the SWP than in the C calves, even though in the finishing phase

Table V. Influence of the substitution of soluble wheat proteins (SWP) for skim milk proteins in milk replacers for veal calves, with or without branch-chain amino acid (BCAA) supplementation, on the calculated dietary intake ($g \cdot j^{-1}$) of essential amino acids truly digestible at the end of the ileum. True digestibility was calculated on the basis of total undigested amino acids minus endogenous undigested with milk amino acids [1]. LSMeans are reported.

Amino acid content in feeds	Control	SWP	SWP+BCAA	SEM[+]	Statistics[++]
Growing phase					
Lysine (Lys)	28.8[a]	29.4[b]	29.4[b]	0.09	T***
Threonine (Thr)	17.1[a]	18.0[b]	18.0[b]	0.05	T***
Valine (Val)	22.0[a]	18.6[b]	23.5[c]	0.07	T***
Leucine (Leu)	33.0[a]	31.9[b]	35.4[c]	0.10	T***
Isoleucine (Ile)	18.6[a]	19.2[b]	20.0[c]	0.06	T***
Arginine (Arg)	11.9[a]	11.7[b]	11.7[b]	0.04	T***
Histidine (His)	8.9[a]	7.7[b]	7.7[b]	0.03	T***
Methionine (Met)	10.1[a]	7.6[b]	7.6[b]	0.03	T***
Phenylalanine (Phe)	17.1[a]	18.7[b]	18.7[b]	0.05	T***
Tyrosine (Tyr)	16.6[a]	11.5[b]	11.3[c]	0.05	T***
Tryptophane (Trp)	4.0[a]	4.3[b]	4.3[b]	0.01	T***
Finishing phase					
Lysine (Lys)	46.2	45.1	44.9	0.39	NS
Threonine (Thr)	28.6[a]	27.7[b]	27.5[b]	0.24	T**
Valine (Val)	34.5[a]	25.3[b]	34.2[a]	0.29	T***
Leucine (Leu)	53.2[a]	44.0[b]	51.6[c]	0.44	T***
Isoleucine (Ile)	30.3[a]	25.9[b]	29.5[c]	0.25	T***
Arginine (Arg)	18.1	17.8	17.7	0.15	NS
Histidine (His)	13.6[a]	11.4[b]	11.3[b]	0.11	T***
Methionine (Met)	15.0[a]	10.8[b]	10.7[b]	0.12	T***
Phenylalanine (Phe)	25.8[a]	26.8[b]	26.6[b]	0.22	T**
Tyrosine (Tyr)	24.6[a]	16.3[b]	16.2[b]	0.19	T***
Tryptophane (Trp)	6.8[a]	7.6[b]	7.5[b]	0.06	T***

[+] SEM: standard error of treatment means ($n = 12$).
[++] T: treatment effect, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS: not significant.

this effect was only noted for collectively housed calves.

The introduction of the SWP concentrate in feeds, with or without BCAA supplementation, resulted in 8 to 18% lower plasma glucose concentrations in the SWP and SWP+BCAA calves compared with the C calves ($P < 0.01$) from the time of the first blood sampling after changes in the diet and during all the growing phase (Fig. 1). During the finishing phase, this effect progressively subsided in the SWP+BCAA calves

Table VI. Blood hemoglobin concentrations ($g \cdot dL^{-1}$), pH and partial pressures in O_2 (pO_2) and CO_2 (pCO_2) measured in calves offered a control diet or diets that included soluble wheat proteins (SWP) without or with branch-chain amino acids (BCAA). LSMeans are reported.

	Control	SWP	SWP+BCAA	SEM[+]	Statistics[++]
Blood hemoglobin					
d84	8.33[a]	9.00[b]	9.38[b]	0.194	T**
d138	7.65	7.91	8.27	0.236	H**
Blood pH					
d84	7.38	7.39	7.37	0.007	NS
d138	7.40	7.41	7.40	0.004	NS
Blood pO2					
d84	27.01	28.43	27.34	0.844	NS
d138	30.64	32.08	30.06	0.744	NS
Blood pCO2					
d84	53.26[a]	49.62[b]	50.64[ab]	0.890	T*
d138	48.27	46.89	48.43	0.598	T × H*

[+] SEM: standard error of treatment means ($n = 12$).
[++] T: treatment effect, H: housing effect, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS: not significant.

whereas it remained significant in the collectively housed SWP calves. These differences could be related to the fact that plasma glucose concentrations increased with time by 36% and 29% in the C ($P < 0.01$) and SWP+BCAA ($P < 0.05$) calves respectively but not in the SWP calves. Insulin levels were 15 to 70% lower ($P < 0.01$) in the SWP and SWP+BCAA calves during the growing phase as compared with the C calves but these differences were no longer significant in the finishing phase (Fig. 1). Insulin concentrations increased 4 to 8 fold with time between d 7 and d 138 ($P < 0.01$) in all treatment groups. Plasma lactate levels did not show any significant differences between the treatment groups and with time (Fig. 1).

The introduction of the SWP concentrate in feeds, with or without BCAA supplementation, also resulted immediately in an approximately 100% increase in the circulating plasma TG and NEFA concentrations ($P < 0.001$) throughout the growing and the finishing phases (Fig. 2). At the end of the growing and the finishing phases, plasma β-hydroxybutyrate concentrations were 45 to 53 and 8 to 38% higher with the SWP and SWP+BCAA treatments respectively ($P < 0.05$) (Fig. 2).

Plasma ammonia concentrations (Fig. 3) increased by 14 to 21% in SWP and SWP+BCAA animals ($P < 0.0001$) until d 126 ($P < 0.0001$). At d 138, plasma ammonia levels in SWP calves were still 6 to 9% higher than those of the C calves ($P < 0.05$). The introduction of the SWP concentrate in the feeds, with or without BCAA supplementation increased plasma urea concentrations during the growing phase by 10 to 32% ($P < 0.05$) (Fig. 3). These differences subsided progressively during the finishing phase and at d 138 plasma urea concentrations were similar across the treatments.

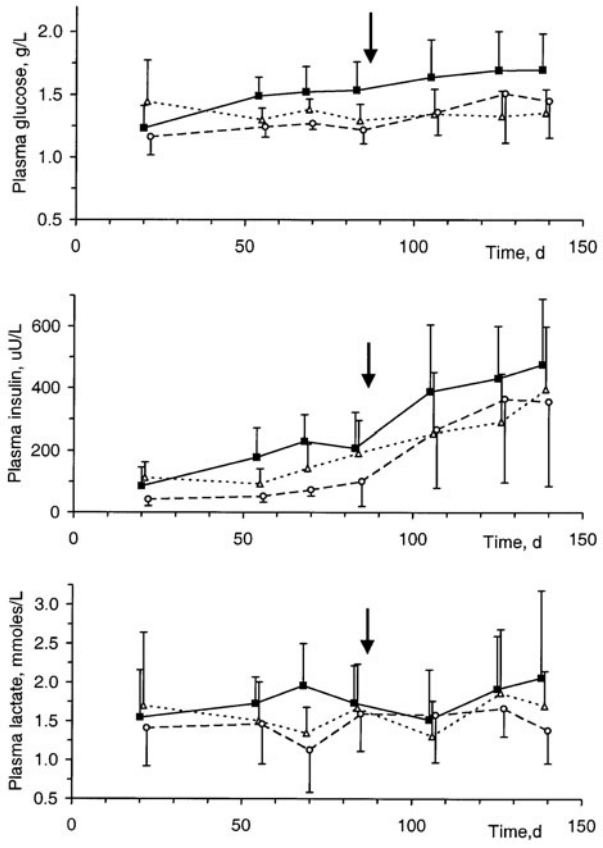

Figure 1. The influence of the substitution of soluble wheat proteins for skim milk proteins in milk replacers, with (○) or without (△) branched-chain amino acid supplementation on plasma glucose, insulin and lactate levels over time. (Control treatment: ■). An arrow indicates the end of the growing phase and the start of the finishing phase.

At d 106, plasma cortisol and T3 levels averaged 1.66, 2.73 and 2.58 ng·mL^{-1} (SEM = 0.403) and 2.07, 2.04 and 2.35 ng·mL^{-1} (SEM = 0.115), for the C, SWP and SWP+BCAA treatment groups respectively (data not shown). No significant differences were noted across the treatments.

3.5. Orientation of muscle energy metabolism

At d 111, total protein content in the *Semitendinosus* muscle did not significantly differ between the treatments. However, total DNA content in the muscle was 16% higher ($P < 0.01$) in the SWP calves than in the C calves (Tab. VII). Consequently, protein/DNA ratio (an indicator of muscle fiber size), was 7.5% lower in the SWP calves (NS).

Activities of muscle oxidative or glycolytic enzymes did not significantly differ between the treatment groups, except for the citrate synthase activity which was 27% higher ($P < 0.05$) in the SWP calves than in the C calves when the results were expressed per g tissue wet weight (Tab. VII). However, the difference was not significant anymore when the results were expressed per mg total protein or per μg total DNA in the muscle

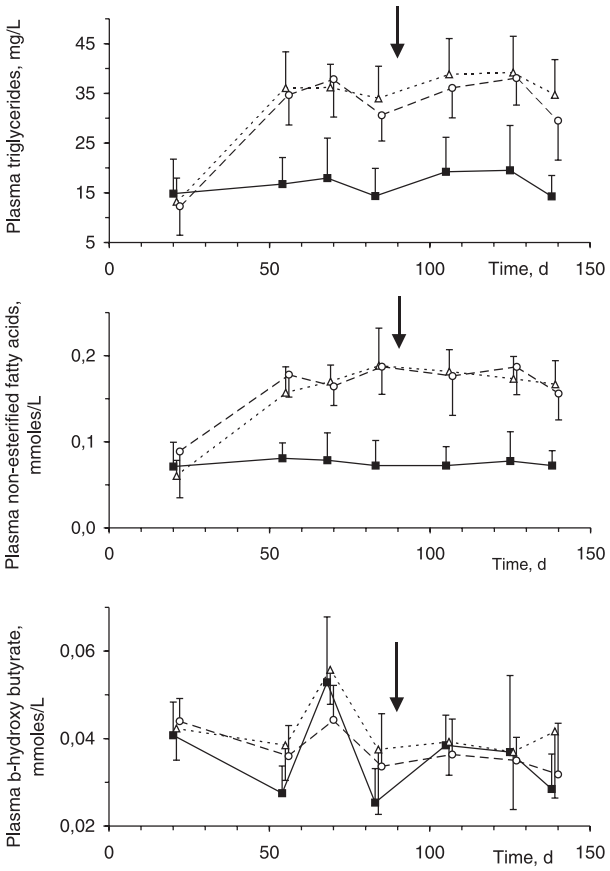

Figure 2. The influence of the substitution of soluble wheat proteins for skim milk proteins in milk replacers, with (○) or without (△) branched-chain amino acid supplementation on plasma triglycerides, non-esterified fatty acids and β-hydroxy butyrate levels over time. (Control treatment: ■). An arrow indicates the end of the growing phase and the start of the finishing phase.

(results not shown). Consequently, CS/LDH or CS/PFK were 16% (NS) and 42% ($P < 0.12$) higher in SWP calves respectively than in the C calves.

In addition, the muscle citrate synthase activity was 23% higher ($P < 0.05$) in calves housed in collective cages than those housed in individual cages, when the results were expressed per g tissue wet weight (5.31 vs. 4.33 units) or per mg total protein (results not shown). This is likely to be due to more spontaneous movements when the calves were housed by groups of two. This difference was lower (11%, $P < 0.10$) when the results were expressed per mg DNA.

3.6. Relationships between growth performances, plasma metabolites and hormones and muscle metabolic characteristics

Of the principal component analysis which was carried out, the first four factors were retained since they showed biological relevance to the work conducted. All together they explained 55% of the standardized variance (21, 13, 12 and 9% respectively). The first factor illustrated the non-clotting characteristics of the SWP containing diets and the link with a more oxidative orientation of muscle energy

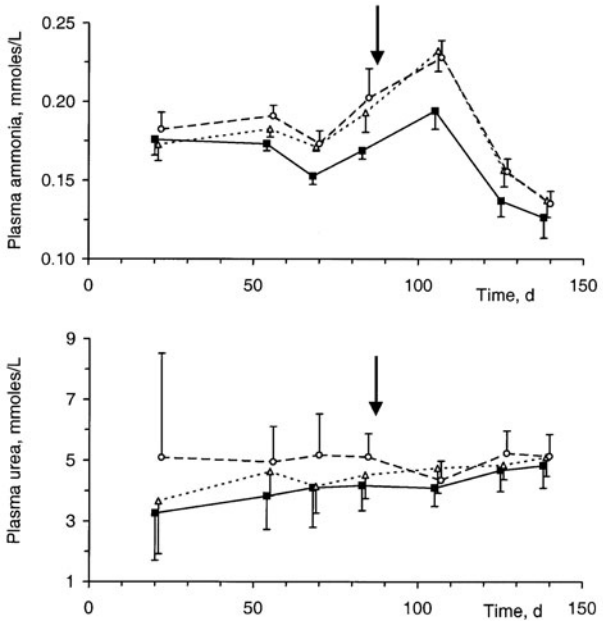

Figure 3. The influence of the substitution of soluble wheat proteins for skim milk proteins in milk replacers, with (○) or without (△) branched-chain amino acid supplementation on plasma ammonia and urea levels over time. (Control treatment: ■). An arrow indicates the end of the growing phase and the start of the finishing phase.

metabolism. It explained almost twice the variance explained by each of the three other factors underlying the importance of the nature of the diet and of the subsequent metabolism of the nutrients. This factor showed positive relationships between plasma NEFA, TG and NH_3 concentrations and the muscle enzymatic ratios CS/LDH and CS/PFK, and the antagonism between these variables and plasma glucose and insulin concentrations (Fig. 4a). This first factor illustrated the positive correlation which existed between the muscle CS/LDH and CS/PFK ratios and plasma NEFA (0.51 and 0.58), TG (0.52 and 0.52) and NH_3 (0.47 and 0.42 respectively) concentrations. When projecting the individual animals along this factor axis, clear discrimination could be seen between the C and SWP containing diets (Fig. 4b), the C animals being characterized by higher insulin and glucose plasma concentrations and lower TG, NEFA and NH_3 plasma concentrations than the SWP animals, as well as by a less oxidative muscle metabolism. The second factor characterized the growth performance and reflected the strong relationship between ADG, carcass weight and body condition score, and the antagonism between these variables and feed efficiency (Fig. 4a). The third factor reflected the color characteristics of the carcasses which was positively related to blood hemoglobin levels, plasma T3 and to a lower extent insulin and glucose but which showed some negative relationship with the ratios of enzymatic activities COX/LDH and COX/PFK, carcass yield and plasma β-hydroxybutyrate concentrations (Fig. 4c). Carcass color was positively correlated with hemoglobin levels (0.70) and plasma T3 concentrations (0.9) and negatively correlated to liver weight (–0.48). In this respect the negative correlation between blood hemoglobin levels and liver weight (–0.55) was notable. When projecting the individual animals along this factor axis, some discrimination could be seen between the housing type. The animals housed in collective housing and characterized by a higher carcass color index, a

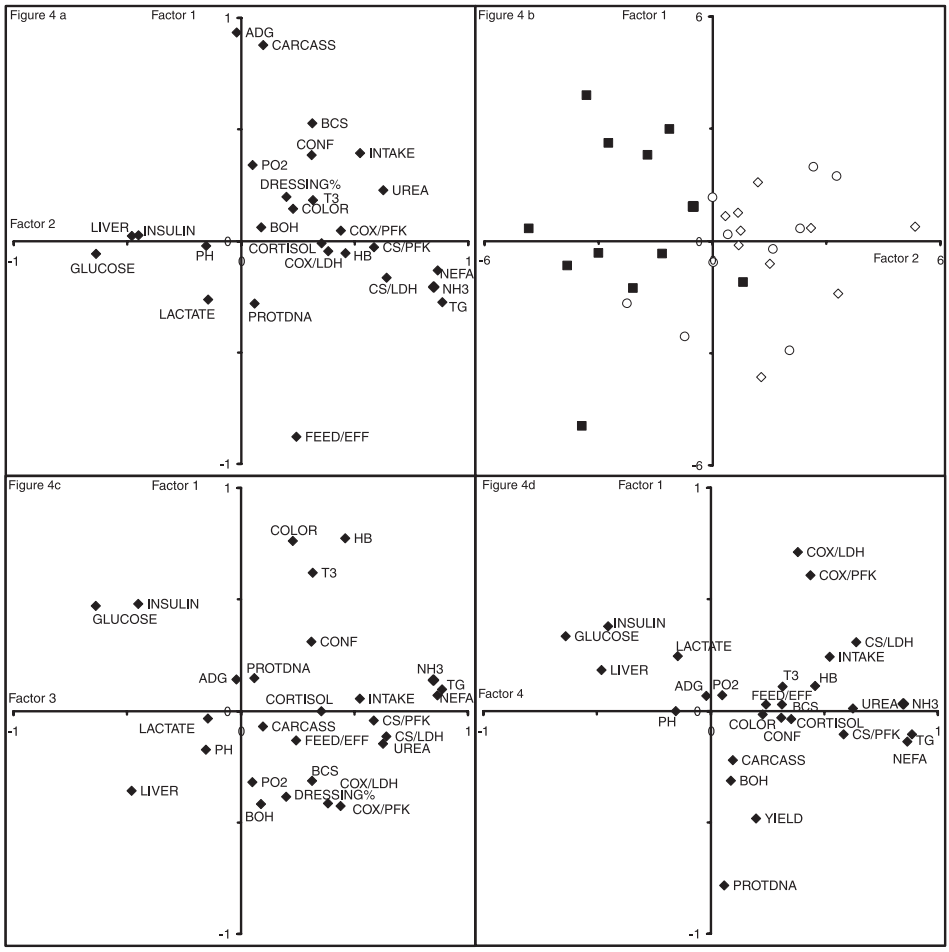

Figure 4. The principal component analyses (PCA) showing (i) relationships between the oxidative orientation of muscle energy metabolism and growth performances (Factors 1 and 2, Fig. 4a), and (ii) relationships between the oxidative orientation of muscle energy metabolism and carcass color (Factors 1 and 3, Fig. 4c) or carcass yield (Factors 1 and 4, Fig. 4d). The PCA also demonstrated individual variability for the treatment control (■), SWP (△) and SWP+BCAA (○) along factor 2 (Fig. 4b). The principal component analyses were performed using the following parameters: (i) average daily gain (ADG), total feed dry matter intake (INTAKE), feed efficiency (FEED-EFF), carcass weight (CARCASS), dressing percentage (DRESSING%), body conformation (CONF), body condition score (BCS), carcass color (COLOR), liver weight (LIVER), (ii) plasma concentrations in glucose (GLUCOSE), insulin (INSULIN), lactate (LACTATE), non-esterified fatty acids (NEFA), triglycerides (TG), β-hydroxy butyrate (BOH), ammonia (NH_3), urea (UREA), tri-iodothyronine (T3) and cortisol (CORTISOL), (iii) blood hemoglobin content (HB), pH (PH) and partial pressure in O_2 (PO2), (iv) ratios between the activities of the muscle enzymes citrate synthase (CS), cytochrome-c-oxydase (COX), phosphofructokinase (PFK) and lactate dehydrogenase (LDH) and (v) the ratio between the muscle protein and DNA contents (PROTDNA).

Table VII. Activity of *Semitendinosus* muscle energy metabolism enzymes measured in calves offered a control diet or diets that included soluble wheat proteins (SWP) without or with branch-chain amino acids (BCAA). LSMeans are reported.

	Control	SWP	SWP+BCAA	SEM[+]	Statistics[++]
Total protein content ($mg \cdot g^{-1}$ tissue wet weight)	157.26	170.06	166.83	6.858	NS
DNA content ($\mu g \cdot g^{-1}$ tissue wet weight)	1690[a]	1967[b]	1732[b]	44.7	T**
Protein/DNA ratio ($mg \cdot \mu g^{-1}$)	0.094	0.087	0.097	0.004	NS
Citrate synthase activity (units$\cdot g^{-1}$ tissue wet weight)	4.29[a]	5.46[b]	4.71[ab]	0.342	T[+], H*
Cytochrome c oxidase activity (units$\cdot g^{-1}$ tissue wet weight)	5.33	6.44	6.15	1.004	NS
Phosphofructokinase activity (units$\cdot g^{-1}$ tissue wet weight)	20.0	18.1	19.6	1.39	NS
Lactate dehydrogenase activity (units$\cdot g^{-1}$ tissue wet weight)	800	844	799	25.3	NS

One unit of enzyme is defined as the amount which, under assay conditions, catalyzes per min the liberation of 1 µmol coenzyme A for citrate synthase, the disappearance of 1 µmol NADH for lactate dehydrogenase and phosphofructokinase, the appearance of 1 µmol NADPH for isocitrate dehydrogenase, and the oxidation of 1 µmol cytochrome c for cytochrome c oxidase.

[+] SEM: standard error of treatment means ($n = 12$).

[++] T: treatment effect, H: housing effect, $^{+}P < 0.10$ (tendency); $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS: not significant.

higher hemoglobin level, a lower liver weight as well as by a more oxidative metabolism were partially discriminated from those housed individually (Fig. 4d). The fourth factor considered (not shown) illustrated a negative relationship between carcass yield and muscle protein/DNA concentration ratio on the one hand and the oxidative orientation of muscle energy metabolism (COX/LDH, COX/PFK) on the other hand. This factor, however, was not very discriminant between the treatment groups.

4. DISCUSSION

In this experiment, the substitution of a mixture of wheat and whey proteins for milk proteins in milk replacers for veal calves allowed the animals to reach similar growth performances as the animals fed conventional milk replacers. This was the case even though the general health status of the animals was not excellent, with the occurrence of respiratory disorders, probably resulting from unstable climatic conditions which influenced the ambient temperature and humidity in the building. Calves receiving the SWP containing diets did not seem to be more fragile than the others. No specific metabolic disorders were detected as shown by the 3 main criteria which were used as indicators of possible disorders (acid-base status, plasma lactate and ammonia concentrations). The plasma ammonia concentrations even though higher with the SWP and SWP+BCAA diets, never reached pathological levels. These results are encouraging for the inclusion of protein substitutes in milk replacers.

The presence of limiting amounts of amino acids in SWP containing diets was assumed to impair growth. In the present experiment, attempts were made to equalize the dietary digestible Lys and Thr contents in all diets, and the dietary Val, Leu and Ile contents in the C and SWP+BCAA diets. The calculated amounts of many essential amino acids which were truly digestible in the intestines still differed among the diets [1]. When expressed relative to the Lys requirements (16 and 14 $g \cdot kg^{-1}$ feed in the growing and finishing phases, respectively), only Val, His and to a much lower extent Ile (in the finishing phase only) would be marginally deficient in the SWP diet. The plasma amino acid levels confirmed that Val was probably the major limiting amino acid with SWP. Despite this, the inclusion of the SWP concentrate in the diet, even in the absence of BCAA supplementation, allowed the calves to reach similar growth and carcass performances as with conventional feeds.

An important aspect of the inclusion of non-clotting proteins in milk replacers is the more rapid gastric emptying previously shown by Toullec and Formal [1] using a SWP concentrate and indicated here by plasma metabolite levels. Indeed, the rise in plasma TG and NEFA concentrations coupled with the drop in glycemia and insulinemia 2.5 h postprandially is clearly characteristic of the ingestion of non-clotting feeds [20–23] and of a lower dietary glucose supply with the SWP containing diets.

The pattern of absorption of digestion end-products influences the utilization of nutrients by the body tissues in preruminant calves [24]. The use of non-clotting diets was also shown to enhance the postprandial amino acid supply, muscle protein synthesis rate [25] thereby possibly increasing the energy demand by peripheral tissues. Thus with the SWP containing diets, a quantitative increase in the utilization of energy-yielding nutrients is expected in the postprandial period itself. In that same period, the contribution of lipids (TG and NEFA) to the energy supply to muscle - appeared here to increase in the first postprandial hours. The use of non-clotting milk replacers also improves insulin sensitivity [25]. In the present experiment, there were suggestions (lower insulinemia and glycemia) that animals fed the non clotting diets showed a less pronounced increase in insulin resistance with age (for a review [26]) than the C calves.

Changes in the balance of available energy-yielding-nutrients may modify the metabolic characteristics of tissues through direct and indirect effects on enzyme activities and the regulation of gene expression (for a review [27, 28]). In the present experiment, changes in the nutrient supply to tissues (measured 2.5 h postprandially) were related to changes in the metabolic characteristics of these tissues (measured in biopsies taken on average 5 h postprandially). Overall, a slight reorientation of muscle energy metabolism towards a more oxidative metabolism was observed when the SWP concentrate was included in the feeds (with or without BCAA supplementation). This effect is probably explained by a large increase in the contribution of lipids to the energy supply to the muscle (for a review [29]) which has been shown to stimulate the oxidative metabolism of muscle, in particular, the activity of the mitochondrial CS enzyme [30]. Additionally citrate, which is synthesized by CS, is a potent inhibitor of PFK thereby directing glucose to glycogen storage rather than to lactate production or mitochondrial oxidation (for a review [29]). It is thus coherent to find the lowest PFK activity in animals which present the highest CS activity. These modifications suggest that glucose would contribute to muscle ATP and lactate synthesis to a lower extent with the SWP containing diets than with the C diet. The number of animals used was probably too small to detect highly significant effects in terms of enzyme activities.

However, the principal component analysis clearly showed the link between the use of non-clotting feeds and the orientation of muscle energy metabolism towards a more oxidative type (factor 1 of the principal component analysis).

The increased oxidative muscle metabolism of animals fed the non clotting diets is also coherent with higher plasma ammonia concentrations which suggests an increased amino acid catabolism in muscles. Indeed, oxidative muscle fibres present a higher protein turnover rate [31]. Additionally in humans, the ingestion of rapidly absorbed whey proteins results in an enhanced postprandial whole body protein synthesis and oxidation rate than those measured following the ingestion of the slowly absorbed casein. Since the oxidation rate is increased to a greater extent than the synthesis rate, this leads to a reduced net ^{13}C-leucine balance during the 7 h following the meal [2, 32]. This suggests that the postprandial protein deposition would be reduced with non clotting diets as compared to clotting ones. The effects in the post-absorptive state and consequences on animal growth performances remain to be evaluated. The only elements of interpretation we have from the present experiment arise from the principal component analysis (factor 4). The protein/DNA ratio (an indicator of muscle fiber size, and hence of muscle growth) and dressing percentage were negatively related to COX/LDH and COX/PFK ratios (indicators of muscle oxidative metabolism) as shown in other growing animals (for a review [27, 28]). Considering that the animal performances did not differ statistically among the treatment groups, it is probable that the modifications in muscle energy and protein metabolism were not sufficiently important to affect muscle growth.

Changes in the muscle metabolism are generally correlated to meat quality. Only the carcass color was measured in the present experiment. In this respect, the results of the principal component analysis (factor 3) demonstrated a clear relation of carcass color with hemoglobin and T3 plasma levels but showed no relationship between carcass color (gathered on factor 3), absorption of nutrients (gathered on factor 1) and a more oxidative muscle metabolism (gathered on factors 1 and 3). This was quite unexpected due to the known stimulation of T3 on mitochondria synthesis and activity, and on the oxidative muscle metabolism (for a review [27]).

An interesting aspect of these data relates to the influence of housing on hematocrit levels in calves and their consequences on carcass color and the orientation of muscle energy metabolism. Higher hematocrit levels have often been observed in collective housing as compared to individual housing [33]. Although not proven, it is possible that the animals which are housed by groups of two, present a generally higher level of physical activity, including the licking of the metal beams which are used as a construction material of the cages; physical activity is known to influence hematocrit levels. The higher physical activity and/or the possible higher intake of iron due to the licking of metal may have favored the orientation of muscle energy metabolism towards the oxidative type (for a review [27]) as demonstrated by the significantly higher citrate synthase activity in the calves housed in collective cages. This effect was even more pronounced than the influence of dietary wheat proteins. Calves housed collectively were also characterized by a less pale meat, which is coherent with the higher muscle oxidative activity. Furthermore, the liver weight was significantly lower in the case of collective housing. The liver is known to be a storage site for iron. In the present case, iron supplementation was similar across the treatment groups but circulating hemoglobin levels were negatively correlated with liver weight, suggesting that the hepatic iron metabolism may have been modified by exercise or uncontrolled iron intake.

5. CONCLUSIONS

When incorporated at a rate of 149 to 167 $g \cdot kg^{-1}$ in the diet, SWP proved to be an interesting alternative to the sole use of whey and skim milk powder as protein sources in milk replacers for veal calves. Growth performances were not modified and no metabolic disorders were noted. Supplementation with BCAA reduced the marginal Val deficiency but did not modify the growth performances. An interesting finding of the present work is that the non-clotting character of the SWP containing diets, as well as the type of housing, were responsible for changes in the orientation of muscle metabolism towards the oxidative type. The consequences of these changes on the metabolic efficiency of nutrient use, especially those of the energy-yielding nutrients and amino acids, need to be investigated as well as the possible consequences on meat quality.

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REFERENCES

[1] Toullec R, Formal M. Digestion of wheat protein in the preruminant calf: ileal digestibility and blood concentrations of nutrients. Anim Feed Sci Technol 1998, 73: 115–130.

[2] Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci USA 1997, 94: 14930–14935.

[3] Longenbach JI, Heinrichs AJ. A review of the importance and physiological role of curd formation in the abomasum of young calves. Anim Feed Sci Technol 1998, 73: 85–97.

[4] Tolman GH, Demeersman M. Digestibility and growth performance of soluble wheat protein for veal calves. In: Metz JHM, Groenestein CM (Ed) New Trends in Veal Calf Production. EAAP Publication, 1991. Vol 52, p 227–233.

[5] Bergmeyer HV, Bernt E, Schmidt F, Stork H. Determination with hexokinase and glucose-6-phosphate. In: Bergmeyer HU (Ed) Methods of Enzymatic Analysis, Academic Press, London, 1974. Vol 3, p 1196–1201.

[6] Gutmann I, Wahlefeld AW. L(+) lactate depermination with lactate dehydrogenase and NAD. In: Bergmeyer HU (Ed) Methods of Enzymatic Analysis. Academic Press, London, 1974. Vol 3, p 1464–1468.

[7] Boissy A, Bouissou MF. Effects of androgen treatment on behavioral and physiological responses of heifers to fear-eliciting situations. Horm Behav 1994, 28: 66–84.

[8] Gutmann I, Bergmeyer HU. Urea and ammonia. In: Bergmeyer (Ed) Methods of Enzymatic Analysis. Academic Press, London, 1974. Vol 3 p 1791–1806.

[9] Williamson DH, Mellanby J. D-3 β hydroxybutyrate. In: Bergmeyer HU (Ed) Methods of Enzymatic Analysis. Academic Press, London, 1974. Vol 4, p 1836–1838.

[10] Itaya K, Ui M. Colorimetric determination of free fatty acids in biological fluids. J Lipid Res 1965, 6: 16.

[11] Picard B, Gagnière H, Geay Y, Hocquette JF, Robelin J. Study of the influence of age and weaning on the contractile and metabolic characteristics of bovine muscle. Reprod Nutr Dev 1995, 35: 71–84.

[12] Bradford MM, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976, 72: 248–254.

[13] Labarca C, Paigen K. A simple rapid and sensitive DNA assay procedure. Anal Biochem 1980, 102: 344–352.

[14] Shepherd D, Garland PB. Citrate synthase from rat liver. Methods Enzymol 1969, 13: 11–16.

[15] Van Hinsberg VWM, Veerkamp JH, Bookelman H. Palmitate oxidation by rat skeletal muscle mitochondria. Comparison of polarographic and radiochemical experiments. Arch Biochem Biophys 1978, 190: 762–771.

[16] Beutler E. Phosphofructokinase. In: Beutler E (Ed) Red Cell Metabolism. A Manual of Biochemical Methods, Grune and Straton Inc., NY, 1971, p 42–44.

[17] Ansay M. Individualité musculaire chez le Bovin: étude de l'équipement enzymatique de quelques muscles. Ann Biol Anim Biochim Biophys 1974, 14: 471–486.

[18] SAS Institute Inc., SAS/STAT User's Guide, Version 6, Fourth Edition, SAS Institute Inc., Cary, NC, USA, 1990.

[19] Patureau-Mirand P, Toullec R, Paruelle JL, Prugnaud J, Pion R. Influence de la nature des matières azotées des aliments d'allaitement sur l'amino-acidémie du veau préruminant. I. Matières azotées du lait du lactosérum, du poisson et des levures d'alcanes. Ann Zootech 1974, 23: 343–358.

[20] Guilloteau P, Paruelle JL, Toullec R, Mathieu CM. Utilisation des protéines par le veau préruminant à l'engrais. Ann Zootech 1975, 24: 243–253.

[21] Durand D, Bauchart D. Variations nycthémérales de la lipémie et de la glycémie au niveau des voies afférentes et efférentes du foie chez le veau préruminant. Reprod Nutr Dev 1986, 26: 371.

[22] Petit HV, Ivan M, Brisson GJ. Digestibility and blood parameters in the preruminant calf fed a clotting or a nonclotting milk replacer. J Anim Sci 1988, 66: 986–991.

[23] Cruywagen CW, Brisson GJ, Tremblay GF, Meissner HH. Effect of curd suppression in a milk replacer on physiological parameters in calves. II. Selected blood profiles. S Afr J Anim Sci 1990, 20: 239–243.

[24] Ortigues I, Martin C, Durand D. Circadian changes in net nutrient fluxes across the portal-drained viscera, the liver and the hindquarters in preruminant calves. J Anim Sci 1996, 74: 895–907.

[25] Grizard J, Toullec R, Guilloteau R, Patureau-Mirand P. Influence de la cinétique d'évacuation gastrique de l'aliment sur l'insulinémie chez le veau préruminant. Reprod Nutr Dev 1982, 22: 475–484.

[26] Bauchart D, Ortigues I, Hocquette JF, Gruffat D, Durand D. Energy and fat metabolism of the liver, the digestive tract and muscles: transport, processing, energy consumption, fixation by tissues. In: Fédération de la Vitellerie Française (Ed) Veal. Perspectives to the Year 2000, Proceedings of the International Symposium, 12–13 September 1995, Le Mans (France), Jouve Mayenne Publ, 1996, p 255–290.

[27] Hocquette JF, Ortigues-Marty I, Pethick D, Herpin P, Fernandez X. Nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat-producing animals. Livest Prod Sci 1998, 56: 115–143.

[28] Hocquette JF, Ortigues-Marty I, Vermorel M. Manipulation of tissue energy metabolism in meat-producing ruminants. Asian Austr J Anim Sci 2001, 14: 720–732.

[29] Randle PJ. Metabolic fuel selection: general integration at the whole-body level. Proc Nutr Soc 1995, 54: 317–327.

[30] Jucker BM, Rennings AJM, Cline GW, Shulman GI. 13C and 31P NMR studies on the effects of increased plasma free fatty acids on intramuscular glucose metabolism in the awake rat. J Biol Chem 1997, 272: 106464–106473.

[31] Reeds P, Davis TA. Hormonal regulation of muscle protein synthesis and degradation. In: Buttery PJ, Boorman KN and Lindsay DB. (Ed) The Control of Fat, Lean Deposition. Butterworths Heinmann, Oxford, 1992, p 1–26.

[32] Boirie Y, Gachon P, Corny S, Fauquant J, Maubois JL, Beaufrère B. Acute postprandial changes in leucine metabolism as assessed with an intrinsically labelled milk protein. Am J Physiol 1996, 271: E1083–E1091.

[33] Bertrand G, Martineau C. Veaux de boucherie élevés en 24 semaines: comparaison case collective de 2 veaux et case individuelle à parois ajourées (normes CEE). Compte-rendu Institut de l'Élevage, n° 95051, 1995.