Aust. J. Biol. Sci., 1981, **34**, 149–59

Bovine α-Lactalbumin C and α_{s1}-, β- and κ-Caseins of Bali (Banteng) Cattle, *Bos (Bibos) javanicus*

K. Bell,[A] *K. E. Hopper*,[B,C] *and H. A. McKenzie*[B,D]

[A] Department of Physiology, University of Queensland, St. Lucia, Qld 4067.
[B] Department of Physical Biochemistry, John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City, A.C.T. 2601.
[C] Present address: Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, N.S.W. 2065.
[D] Address for reprints.

Abstract

An electrophoretic examination is made of milk samples taken from eight Bali (banteng) cattle, *Bos (Bibos) javanicus*, at Beatrice Hills, Northern Territory, Australia. Starch-gel electrophoresis at pH 8·5 ($NaOH–H_3BO_3$ buffer) and filter-paper electrophoresis at pH 8·6 (diethylbarbiturate buffer) indicate that all samples contain a new α-lactalbumin variant, designated α-lactalbumin C. The order of mobility for bovine variants is A > B > C. The C variant differs from the common B variant in having one more amide residue (substitution of Asn for Asp or Gln for Glu).

Examination of milk samples by urea–starch-gel electrophoresis at alkaline pH indicates that there is a new α_{s1}-casein variant, designated α_{s1}-casein E_{Bali}, present in some samples. No new κ-casein variant is detected by this method (all samples typing as κ-casein B). A new variant of β-casein, designated A^4, is detected by urea–starch-gel electrophoresis at low pH.

The variants of milk proteins observed in this paper and in Bell *et al.* (1981) are discussed in relation to those of other members of the Bovinae, especially the yak, *Bos (Poephagus) grunniens*.

Introduction

In the paper by Bell *et al.* (1981) we have examined samples of milk taken from eight Bali (banteng) cattle, *Bos (Bibos) javanicus*, at the Beatrice Hills Experiment Station, Northern Territory. It was shown that β-lactoglobulin is the dominant whey protein present and that it occurs as three new variants, designated β-lactoglobulin E, F and G. In the course of this work it was noticed that there was a prominent band due to α-lactalbumin, moving more slowly than bovine α-lactalbumin B of *Bos taurus* or the A variant of *Bos indicus*. The isolation and characterization of this new variant, designated α-lactalbumin C, is described in the present paper. Attention is also given to the variants of α_{s1}-, β- and κ-casein. Two new variants, α_{s1}-casein E_{Bali} and β-casein A^4 are detected. In the light of these studies on the milk proteins of the subgenus *Bos (Bibos) javanicus* and the work of Grosclaude *et al.* (1976) on those of the subgenus *Bos (Poephagus) grunniens*, it is possible to compare the variants of the proteins of these subgenera to the known variants of the subgenus *Bos* and to those of the genus *Bubalus*, and consider their evolution.

Materials and Methods

These were similar to those used by Bell *et al.* (1981), with the following additions. Filter-paper electrophoresis of total 'whey' protein was performed according to the method of Aschaffenburg and Drewry (1955). Urea–starch-gel electrophoresis in alkaline solution was performed as described by Aschaffenburg and Thymann (1965). β-Caseins were further typed in acid solution (formic

acid buffer, pH 3·0) by the method of Bell described by McKenzie (1971, p. 500). Starch-gel electrophoresis of α-lactalbumin fractions was performed with the semi-discontinuous buffer system of Ferguson and Wallace (1963).

Results

Electrophoretic Typing of α-Lactalbumin

All skim milk samples of the eight Bali (banteng) cows were examined by the starch-gel electrophoresis procedure for typing β-lactoglobulin, as described by Bell *et al.* (1981), and showed a single α-lactalbumin band, moving more slowly than the A and B variants (see Fig. 1 of that paper). This band had a lower mobility than bovine serum albumin in this buffer system, whereas the A and B variants had a higher mobility than bovine serum albumin. That the protein in the band was an α-lactalbumin was confirmed by immunoelectrophoresis in which a single arc was formed with rabbit antiserum to bovine α-lactalbumin B. Also, the new protein and α-lactalbumin A and B showed immunological identity on immunoelectrophoresis with rabbit antiserum to the B variant. The new variant has been designated α-lactalbumin C.

The C variant also showed a single band moving more slowly than the A and B variants on filter-paper electrophoresis at pH 8·6 (Fig. 1).

Isolation of α-Lactalbumin C

A sample of ammonium sulfate total whey protein from cow 171 was dialysed against 0·056 M Tris–0·05 M HCl buffer, pH 7·8, and subjected to gel filtration on Sephadex G75. The elution profile was similar to that of Fig. 2 in Bell *et al.* (1981). The minor fraction 4 exhibited two bands on electrophoresis. They were of lower mobility than the main α-lactalbumin C fraction. These bands probably represent components analogous to the minor glycoprotein components isolated from fraction 4 during studies of bovine α-lactalbumin A and B (Hopper and McKenzie 1973). They have not been studied further in the present work.

Fraction 5 (Bell *et al.* 1981) contained the main α-lactalbumin C component, as well as a minor band analogous to the fast component (FC) of α-lactalbumin A and B (Hopper and McKenzie 1973). This fraction was concentrated by ultrafiltration and subjected to ion-exchange chromatography on DEAE-Sephadex A50 in the Tris–HCl buffer and eluted with a linear NaCl gradient. The elution profile showed one major peak (the main α-lactalbumin C component), followed by two minor peaks. The first minor peak was probably a reflection of apparent heterogeneity (Hopper 1973) and the second minor peak was component FC of α-lactalbumin C. The mobility of the latter component was similar to that of the main component of bovine α-lactalbumin B on electrophoresis in the Ferguson–Wallace system. It will be recalled that, in turn, FC of α-lactalbumin B has the same mobility as the main component of α-lactalbumin A (Hopper and McKenzie 1973). Electrophoretic patterns of the variants A, B and C are compared in Fig. 2 of the present paper.

The elution volume of α-lactalbumin C on gel filtration was the same as that for the B variant, and since it does not contain carbohydrate (see below), it probably has a similar molecular weight to that of α-lactalbumin B (*c.* 14000).

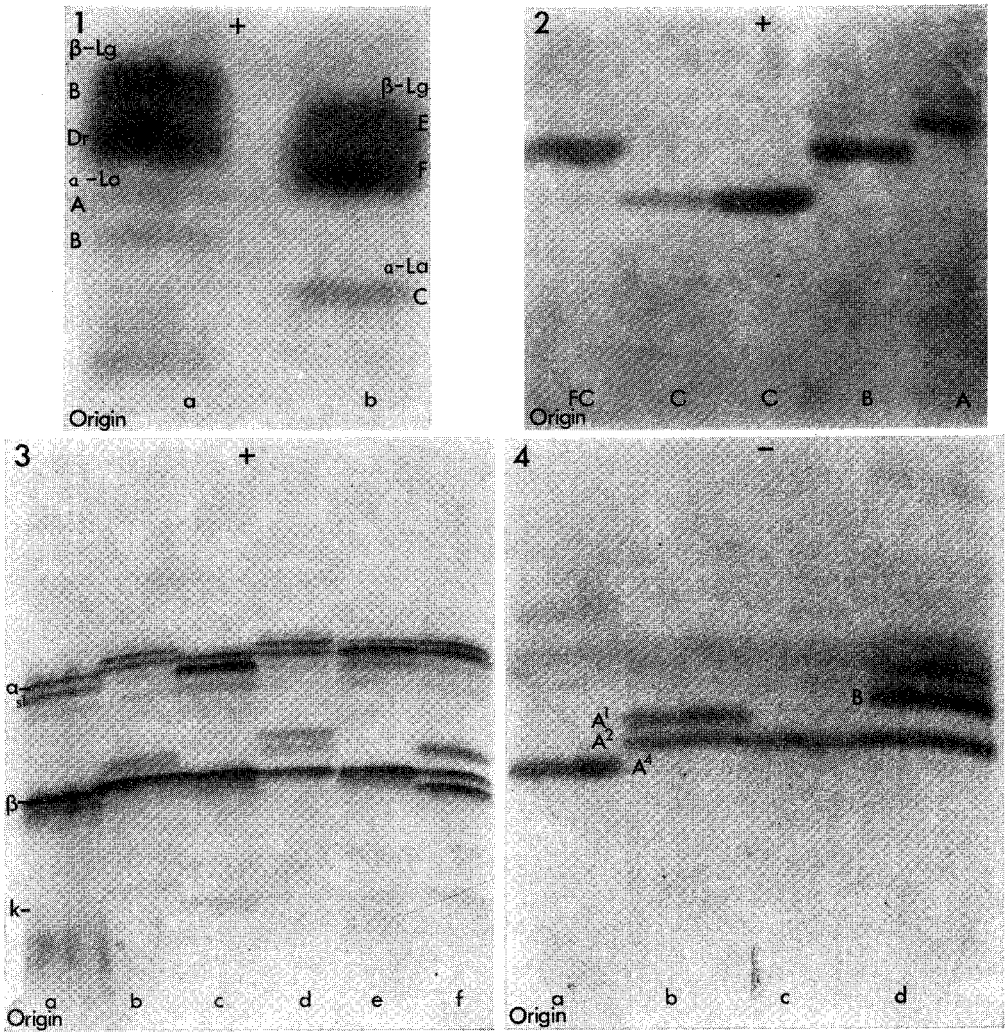

Fig. 1. Paper electrophoretic patterns of concentrated 'whey' protein solutions in diethylbarbiturate buffer, pH 8·6, ionic strength 0·05. (*a*) Droughtmaster: β-lactoglobulin BDr and α-lactalbumin AB; (*b*) Bali (banteng): β-lactoglobulin EF and α-lactalbumin C.

Fig. 2. Starch-gel electrophoretic patterns of α-lactalbumin variants in Ferguson–Wallace semi-discontinuous buffer system. FC, α-lactalbumin C, fast component; C, α-lactalbumin C at two different concentrations; B, α-lactalbumin B; A, α-lactalbumin A.

Fig. 3. Alkaline urea–starch-gel electrophoretic patterns, Aschaffenburg–Thymann system, showing casein types in skim milk samples. (*a*) Bali cow: α_{s_1}-casein CE, β-casein A, κ-casein B; (*b*) Droughtmaster: α_{s_1}-casein BC, β-casein A, κ-casein A; (*c*) Bali: α_{s_1}-casein CE; β-casein A; κ-casein B; (*d*) Droughtmaster: α_{s_1}-casein BC, β-casein A, κ-casein B; (*e*) Bali: α_{s_1}-casein CE, β-casein A, κ-casein B; (*f*) Droughtmaster: α_{s_1}-casein BC, β-casein AB, κ-casein B. β-lactoglobulins A, B and Droughtmaster migrate just ahead of the β-casein bands in patterns (*b*), (*d*), and (*f*).

Fig. 4. Acid urea–starch-gel electrophoretic patterns of skim milk samples (system of Bell). (*a*) Bali: β-casein A^4; (*b*) Droughtmaster: β-casein A^1,A^2; (*c*) Bali: β-casein A^2; (*d*) Droughtmaster: β-casein BA^2.

Amino Acid Analysis and Peptide Maps

Initial amino acid analyses of the main component and fast component (FC) of α-lactalbumin C are presented as set 1 in Table 1. There is no apparent difference in the analyses. This is accordance with expectation if the FC and main components of the C variant are related to one another in the same way as the FC and main components of each of the previously studied variants (A and B) are related to one another. In these variants FC differs from the main component in having one less amide residue per molecule: such a difference would not be detected in amino acid analysis (Hopper and McKenzie 1973). No humin formation was observed in the hydrolysates and no hexosamines were detected in the analyser elution profiles. Hence it would appear that neither component contains a carbohydrate moiety.

Table 1. Amnio acid analysis of bovine α-lactalbumin variants C (fast component), C, B and A
Results are expressed as moles per mole protein. n.d., not determined

Residue	Set 1[A]		Set 2[B]		
	Variant C (fast component)	Variant C (main component)	Variant C	Variant B	Variant A
Lys	13·0	12·9	12·1	12·1	12·3
His	2·9	2·9	3·0	2·5	2·6
Arg	0·9	0·8	1·0	1·0	0
Asp	20·8	21·2	20·6	20·5	20·8
Thr	6·8	6·7	6·8	6·8	6·8
Ser	6·2	5·9	6·8	6·9	7·0
Glu	12·6	12·8	12·9	13·1	14·4
Pro	2·3	1·7*	2·1	2·1	2·2
Gly	6	6	6	6	6
Ala	3·2	2·8	3·3	3·0	3·4
½Cys	—	7·8*	—	—	—
Val	5·4	5·6	5·8	5·8	5·8
Met	0·8	0·8	0·9	0·9	1·0
Ile	7·3	7·6	8·0	8·1	8·2
Leu	12·3	12·6	13·1	13·3	13·3
Tyr	3·5	3·6	3·9	4·1	3·9
Phe	3·6	3·7	4·0	4·0	4·1
Trp	n.d.	4·2	n.d.	n.d.	n.d.

[A] Analysis of variant C (fast component) and variant C (main component) run in parallel on a Beckman 120B Amino Acid Analyzer with two-column procedure. Values for the fast component are of a single analysis after hydrolysis for 22 h and for the main component are means of two analyses except where indicated below. Ser and Thr based on extrapolated values after hydrolysis for 22, 48 and 72 h. The same correction applied to Ser and Thr for the fast component. Pro and Cys analyses (marked with asterisk) for the main component are single analyses on an oxidized sample. Pro for the fast component is corrected value for a non-oxidized sample. All values are given on the basis of Gly = 6, the value for other known bovine α-lactalbumins.

[B] Analyses of variants A, B and C run in parallel on modified 120C amino acid analyser with single-column procedure (1 nanomole sensitivity) and improved hydrolysis procedure and reagents. Values are means of two analyses, as described in text. Trp was determined by the ultraviolet absorption method.

The results for the analysis of the C variant were compared with our previous analyses of A and B variants and with the composition of the B variant based on its known sequence. It was tentatively concluded that the C variant differs from the

B variant in having +1 Lys and probably −1 Ser. This difference was stated with some reservations because the serine result was based on an extrapolation procedure, and there had been occasional difficulties with base-line levels for lysine. Also the A and B analyses were not performed in parallel with the C analyses on the same day. Furthermore the substitution Lys/Ser involves two base changes in nucleic acid codons.

The analyses of A, B and C variants were repeated recently, using improved procedures (see Bell *et al.* 1981 and footnote B to Table 1). Duplicate analyses were performed in parallel on the same day, together with analyses of bovine β-lactoglobulin B, E and F. Values of Thr, Ser, Ile, Pro, His, Met and Tyr were corrected on the basis of the known β-lactoglobulin compositions. The results are shown as set 2 in Table 1.

There are no differences in amino acid analyses between α-lactalbumin B and C. The A variant shows the known difference from B of +1 Gln, −1 Arg (and in turn from C). Again there was no evidence of humin formation during hydrolysis, and hexosamine was absent. Thus the charge difference between C and B must lie in an amide difference (Asn for Asp and/or Gln for Glu).

Table 2. Number of amino acid residues in bovine α-lactalbumin variants A, B and C per molecule of 123 residues (molecular weight *c.* 14200)

Values for B are from the amino acid sequence of Brew *et al.* (1970). Values for A are studies of Bell *et al.* (1970*a*). Values for C are based on present work. The order in which the amino acid residues are listed in this table is on the same basis as that of Table 5 in Bell *et al.* (1981)

Residue	Variant A	Variant B	Variant C	Residue	Variant A	Variant B	Variant C
Asp+Asn	21	21	21	½Cys	8	8	8
Glu+Gln	14	13	13	Met	1	1	1
His	3	3	3	Val	6	6	6
Arg	0	1	1	Leu	13	13	13
Lys	12	12	12	Ile	8	8	8
Gly	6	6	6	Pro	2	2	2
Ser	7	7	7	Phe	4	4	4
Thr	7	7	7	Tyr	4	4	4
Ala	3	3	3	Trp	4	4	4

A careful examination of mobilities of α-lactalbumin variants in gel and paper electrophoresis of skim milk samples and in gel electrophoresis of isolated α-lactalbumins (A, B and C) indicates that only one amide residue should be involved. Recent tryptic peptide mapping of the A, B and C variants (D. C. Shaw, unpublished results) gave some difference peptides. The results of the analyses are not completely clear cut, but it seems that there is an Asn/Asp substitution. Further peptide studies are in progress. The number of amino acid residues considered to be present in each of the three variants is shown in Table 2.

Electrophoretic Typing of Caseins

α_{s1}-Casein

Electrophoretic patterns of samples in the alkaline starch-gel system showed either the α_{s1}-casein C band or the C band together with another band of equal intensity

in the α_{s1}-region. The latter band moved more slowly than any then known α_{s1}-casein and was designated α_{s1}-casein E. Typical patterns are shown in Fig. 3.

κ-Casein

A single κ-casein variant, having the same mobility as the common B variant was found in all samples examined (see Fig. 3).

β-Casein

All the samples gave rise to a single band corresponding to β-casein A in the alkaline gel system (see Fig. 3). However, in the acidic gel system designed to type the variants A^1, A^2, A^3, it was found that either A^2 alone was present or A^2 in company with a band of equal intensity, moving at a slightly slower rate than A^2 (see Fig. 4). In a separate gel (not shown) it was found that the latter variant had a slightly lower mobility than the rare bovine A^3 variant. Hence this new variant was designated β-casein A^4.

At present no opportunity has been available to study further these variants.

Discussion

The samples of milk of Bali (banteng) cattle that have been available to us for study have been prolific in new milk protein variants (see Table 3). The major whey protein, β-lactoglobulin, occurs as three new variants: E, F and G (Bell *et al.* 1981). None of the common variants have been found. However, it is not possible to conclude that they are generally absent from the milk of *Bos javanicus* cattle until samples from a wider range of animals have been examined.

Table 3. Summary of protein types in milk samples from Bali (banteng) cattle

—, not determined

Cow No.	Whey proteins		Caseins		
	β-Lactoglobulin	α-Lactalbumin	α_{s1}-	β-	κ-
150	EF	C	C	A^2	B
158	EF	C	—	—	—
159	F	C	C	A^2	B
166	E	C	C	A^2	B
169	E	C	—	—	—
170	E	C	C	A^2	B
171	EF	C	CE	A^4	B
454	EF	C	CE	A^2,A^4	B

All samples contained only a single major α-lactalbumin (designated C) of lower mobility than the A and B variants. The α_{s1}-caseins occurred either as α_{s1}-casein C, or as the C variant together with a new variant α_{s1}-casein E. The samples all contained a new β-casein variant, A^4.

Since we originally observed the new variants in the milk of Bali cattle in 1970–71, Grosclaude *et al.* (1976) have published studies of the milk proteins of 156 cattle, including 42 yaks (*Bos grunniens*), in a high Nepalese valley. The samples from these yaks contained only one β-lactoglobulin, designated D_{yak}, which appears to be identical with β-lactoglobulin E (Bell *et al.* 1981). Only one other new milk protein variant was found in these cattle: a new α_{s1}-casein designated α_{s1}-casein E.

This variant is possibly the same as the E variant we have observed in the milk of Bali cattle. Until their chemical nature is further studied it is suggested the Bali and yak variants be tentatively identified as α_{s1}-casein E_{Bali} and E_{yak} respectively.

It is now possible to compare the variants of the milk proteins of the subgenus *Bos* with two other subgenera: *Bibos* and *Poephagus*. The present known variants and their occurrence are summarized in Table 4.

Table 4. Genetic variants of milk proteins of the genus *Bos*

Data based on the work of Aschaffenburg (1968), McKenzie (1970, 1971), Voglino (1972), Grosclaude *et al.* (1976), Bell *et al.* (1981) and the present work

Protein	Occurrence of variant				
	Bos taurus		*Bos indicus*	*Bos (Bibos) javanicus*	*Bos (Poephagus) grunniens*
	All breeds	Less common		Bali	Yak
α_{s1}-Casein	B (high), C	A (Friesian) D (Flamande)	B, C (high)	C, E_{Bali}	C (high) E_{yak}, B (rare)
β-Casein	A^2 A^1 (most breeds)	A^3 (rare)	A	A^2,A^4	A^2
	B (most breeds)	C (rare—Guernsey) E (Piedmont)	B_z (low) C (rare—Deshi, Boran)		
κ-Casein	A,B		A,B	A,B	A (high), B
β-Lactoglobulin	A,B	C (Jersey) D (rare)	A,B Droughtmaster (cross)	E,F,G,	D_{yak} (probably equiv. to E)
α-Lactalbumin	B		A,B	C	B

Before discussing these variants it is of interest to consider the origin of the Bali cattle in Australia from which we obtained our samples. It is known, as stated in Bell *et al.* (1981), that the cattle were first brought from Indonesia to the military settlements of northern Australia over a century ago. It was previously considered (Calaby 1975) that these cattle arrived on the Cobourg Peninsula from Bali in 1849. However, Letts (1979) has recently concluded that 'the breed had been in the peninsula at least since 1845'. Balinese cattle are a domestic form of *Bos (Bibos) javanicus*. They are found in Bali, Lombok, Timor, South Celebes, East Java and Borneo. The wild form (sometimes called *Bos banteng*) is found in Malaysia and Indonesia. There seems little doubt that the common domestic cattle of Indonesia (excluding water buffalo) are of Indian origin, Zebu having been imported since the middle ages (see, for example, Raffles 1817 and Meijer 1962).

While there is considerable, but by no means complete, knowledge of the domestication of the subgenus *Bos*, and of the origin of the present domestic forms of *Bos primigenius*, little is known of the origin of *Bos (Poephagus) grunniens* or of *Bos (Bibos) javanicus* (Zeuner 1963*a*, 1963*b*; Epstein 1974; Bökönyi 1976). A summary is given in Fig. 5 of what we believe is a reasonable view of the relationships of the Bovinae based on archeological and genetic studies and their physical characteristics. The occurrence of the variants summarized in Table 4 may be examined in the light of the relationships of Fig. 5.

Grosclaude *et al.* (1976) consider that β-lactoglobulin D_{yak} and α_{s1}-casein E are probably specific to *Bos grunniens*. However, we have concluded that the amino acid sequence β-lactoglobulin E is identical to that of β-lactoglobulin D_{yak}. Also it

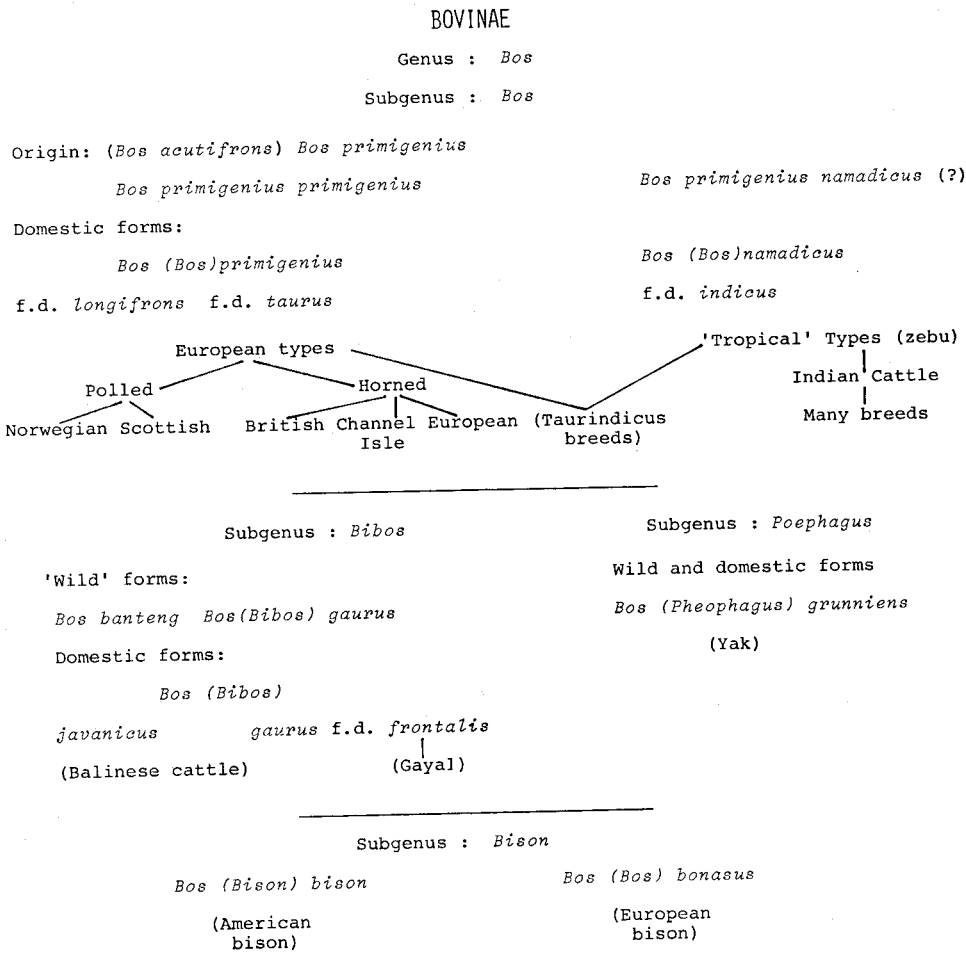

Fig. 5. Schematic representation of the relationships of the members of the *Bos* genus ('true cattle') of the Bovinae. The *primigenius* breeds probably all developed from a single wild ancestral form *Bos primigenius* (the aurochs), although there is some disagreement as to whether *Bos acutifrons* (shown in parenthesis) was a separate form (see, for example, Zeuner 1963*a*, 1963*b*). Some polled cattle may have developed from the extinct *Leptobos* (not shown) (see Kelley 1960 and Zeuner 1963*b*). The nomenclature is based on that summarized by Zeuner (1963*b*). The name *Bos* (*Bos*) *namadicus* f.d. *inidicus* has been used for the zebu rather than *Bos* (*Bos*) *primigenius* f.d. *indicus* as used by Zeuner (1963*b*). The former name would seem to be more in accord with present views on their origin (see the discussion of Francis 1965). However, we have used Zeuner's name *Bos primigenius namadicus* for the wild form, rather than *Bos namadicus*, because the relationship to *primigenius* cattle is not unequivocally resolved. (The Indian or water buffalo and the African buffalo are considered to be members of other genera: *Bubalus* and *Syncerus* respectively.) f.d. signifies *forma domestica* (domestic form).

seems that α_{s1}-casein E of the yak is the same as α_{s1}-casein E of Bali cattle. It is unlikely that Bali cattle have cross-bred with the yak. Thus the similarities in variants of some of the milk proteins may reflect an ultimate common origin and subsequent

divergence, or may merely be the result of independent development. Grosclaude *et al.* (1976) present some evidence, from the high frequency of the allelic combination α_{s1}-casein C-β-casein A^2-κ-casein A in *Bos grunniens*, that tends to make them closer to *Bos indicus* than *Bos taurus*. While there is insufficient data available for the Bali cattle there is some indication from the casein variants that *Bos javanicus* may also be closer to *Bos indicus* than *Bos taurus*.

The new α-lactalbumin variant C we have found in the milk of Bali cattle has not hitherto been detected in any other type of cattle. The same appears to be true of the β-casein A^4 variant. It remains to be seen if either of these variants can be used as markers for *Bos javanicus*.

The β-lactoglobulin$_{\text{Droughtmaster}}$ variant has only been found in the milk of Droughtmaster cattle. This breed consists of approximately half Brahman (zebu, *Bos indicus*) and approximately half European breeds (mainly Shorthorn, *Bos taurus*) (Bell *et al.* 1970*b*). We believe the variant arises from the zebu component and that the variant will be detected in the milk of 'pure' *Bos indicus* cattle if electrophoretic methods capable of resolving this variant are used in typing large numbers of these animals. Whether the variant will be found only in zebu crosses remains to be seen.

The β-lactoglobulin C variant has been identified only in Jersey cattle (Aschaffenburg 1968; McKenzie 1970). It has not been found in the other Channel Island breeds. However, β-casein C has been found only in the Guernsey. It is not yet certain that these two variants are exclusive to these breeds respectively.

Only two κ-casein variants (A and B) have been found and they are common to all breeds and subgenera of *Bos* that have so far been investigated.

The α-lactalbumin B variant is the only α-lactalbumin variant so far found in *Bos taurus*. Both the B and A variants have been found in *Bos indicus* and the Droughtmaster (*Bos indicus* × *Bos taurus*) (Blumberg and Tombs 1958; Bhattacharya *et al.* 1963; Aschaffenburg 1968; Bell *et al.* 1970*a*). In filter-paper electrophoretic examination of milk samples from the Indian water buffalo, Bhattacharya *et al.* (1963) found an α-lactalbumin with identical mobility to that of α-lactalbumin A, and a β-lactoglobulin with similar mobility to that of bovine β-lactoglobulin B. The water buffalo is not a member of the *Bos* genus, but is of the genus *Bubalus*. In a subsequent investigation of these proteins isolated from the Italian water buffalo (*Bubalus arnee*), Addeo *et al.* (1976) showed these proteins were not identical with the corresponding proteins of the genus *Bos*. Buffalo β-lactoglobulin differs from the bovine B variant by Ile/Leu at position 1, Val/Ile at position 162 and Gln/Ile at an unknown position.

Addeo *et al.* (1976) showed that, while the buffalo α-lactalbumin has the same mobility as the bovine A protein, it does not have the zero arginine content characteristic of the A protein. It more closely resembles the B variant in having one arginine residue per molecule. Buffalo α-lactalbumin differs from bovine B by an Asn/Gly substitution at position 17 and a Glu/Gln or Asp/Asn substitution at an unknown position. The latter substitution would account for it having the same mobility as the bovine A variant. These results indicate the danger of assuming the identity of variants on the basis of identical electrophoretic mobility. The genera *Bos* and *Bubalus* diverged towards the middle of the Miocene period. The results of the French workers would tend to indicate that the B variant of bovine β-lactoglobulin is the wild type of the *Bos* genus.

In summary, the variants of the milk proteins of the Bovinae so far discovered are a reflection at the molecular level of the common origin and parallel development of the genera *Bubalus* and *Bos* and the subgenera of the genus *Bos*. It is doubtful if any one variant within the genus *Bos* can be regarded as exclusive either to a subgenus or breed. Further light will only be thrown on this problem when more protein polymorphism results are obtained from adequate numbers of both rare and common breeds of cattle.

Acknowledgments

Acknowledgments are similar to those of Bell *et al.* (1981), with additional thanks being due to D. C. Shaw for permission to quote unpublished work.

References

Addeo, F., Mercier, J.-C., and Ribadeau-Dumas, B. (1976). Eléments de structure primaire de l' α-lactalbumine et de la β-lactoglobuline de buffle. *FEBS Lett.* **63**, 255–9.

Aschaffenburg, R. (1968). Genetic variants of milk protein: their breed distribution. *J. Dairy Sci.* **38**, 447–60.

Aschaffenburg, R., and Drewry, J. (1955). Occurrence of different beta-lactoglobulins in cow's milk. *Nature* (*London*) **176**, 218–20.

Aschaffenburg, R., and Thymann, M. (1965). Simultaneous phenotyping procedure for the principal proteins of cow's milk. *J. Dairy Sci.* **48**, 1524–5.

Bell, K., Hopper, K. E., McKenzie, H. A., Murphy, W. H., and Shaw, D. C. (1970*a*). A comparison of bovine α-lactalbumin A and B of Droughtmaster. *Biochim. Biophys. Acta* **214**, 437–44.

Bell, K., McKenzie, H. A., Murphy, W. H., and Shaw, D. C. (1970*b*). β-lactoglobulin$_{\text{Droughtmaster}}$: A unique protein variant. *Biochim. Biophys. Acta* **214**, 427–36.

Bell, K., McKenzie, H. A., and Shaw, D. C. (1981). Bovine β-lactoglobulin E, F and G of Bali (banteng) cattle, *Bos* (*Bibos*) *javanicus*. *Aust. J. Biol. Sci.* **34**, 133–47.

Bhattacharya, S. D., Roychoudhury, A. K., Sinha, N. K., and Sen, A. (1963). Inherited α-lactalbumin and β-lactoglobulin polymorphism in Indian zebu cattle. Comparison of zebu and buffalo α-lactalbumins. *Nature* (*London*) **197**, 797–9.

Blumberg, B. S., and Tombs, M. P., (1958). Possible polymorphism of bovine α-lactalbumin. *Nature* (*London*) **181**, 683–4.

Bökönyi, S. (1976). Development of early stock rearing in the Near East. *Nature* (*London*) **264**, 19–23.

Brew, K., Castellino, F. J., Vanaman, T. C., and Hill, R. L. (1970). The complete amino acid sequence of bovine α-lactalbumin. *J. Biol. Chem.* **245**, 4570–82.

Calaby, J. H. (1975). Introduction of Bali cattle to Northern Australia. *Aust. Vet. J.* **51**, 108.

Epstein, H. (1974). Yak and chauri. *World Anim. Rev.* **9**, 8–12.

Ferguson, K. A., and Wallace, A. L. C. (1963). The characterization of pituitary hormones by starch gel electrophoresis. *Recent Prog. Horm. Res.* **19**, 1–55.

Francis, J. (1965). Definition and use of zebu, Brahman or *Bos indicus* cattle. *Nature* (*London*) **207**, 13–16.

Grosclaude, F., Mahé, M.-F., Mercier, J. C., Bonnemaire, J., and Teissier, J. H. (1976). Polymorphisme de lactoprotéines de bovinés népalaise. *Ann. Genet. Sel. Anim.* **8**, 461–79.

Hopper, K. (1973). Apparent heterogeneity of bovine α-lactalbumin A and B in DEAE-Sephadex chromatography. *Biochim. Biophys. Acta* **293**, 364–70.

Hopper, K., and McKenzie, H. A. (1973). Minor component of bovine α-lactalbumin A and B. *Biochim. Biophys. Acta* **295**, 352–63.

Kelley, R. B. (1960). 'Principles and Methods of Animal Breeding.' 3rd Ed. pp. 1–7 (Angus and Robertson: Sydney.)

Letts, G. A. (1979). 'Feral Animals in the Northern Territory.' Report of the Board of Inquiry, Northern Territory Government. Papers submitted to the Inquiry by P. Spillet, p. 43. (Northern Territory Govt. Printer.)

McKenzie, H. A. (1970). Some recent advances in the chemistry of milk proteins. Proc. XVIII Int. Dairy Congr. Sydney. Vol. 2. pp. 187–205. (XVIII Int. Dairy Congr.–Aust. Nat. Dairy Committee.)

McKenzie, H. A. (1971). 'Milk Proteins.' (Ed. H. A. McKenzie.) Vol. II. (Academic Press: New York.)

Meijer, W. C. (1962). 'Das Balirind.' (A. Ziemsen: Wittenberg, Germany.)

Raffles, T. S. (1817). 'The History of Java.' 2 volumes. (Black, Parbury and Allen: London.)

Thompson, M. P. (1970). Phenotyping milk proteins: a review. *J. Dairy Sci.* **53**, 1341–8.

Voglino, G. F. (1972). A new β-casein variant in Piedmont cattle. *Anim. Blood Grps Biochem. Genet.* **3**, 61–2.

Zeuner, F. E. (1963*a*). 'A History of Domesticated Animals.' pp. 201–55. (Harper and Row: New York.)

Zeuner, F. E. (1963*b*). The history of domestication of cattle. In 'Man and Cattle'. (Eds A. E. Mourant and F. E. Zeuner.) pp. 9–15, with nomenclature on p. 159. (R. Anthrop. Inst. Gt Brit. and Ireland: London.)

Manuscript received 17 September 1980, accepted 26 November 1980