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Factors affecting colostrum quality and calf passive transfer levels in Holstein cattle

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The aim of this study was to determine the quality of colostrum in Holstein cattle and factors affecting serum total protein and immunoglobulin levels of calves. In the study colostrum samples taken from 334 Holstein cows in the first milking and serum samples collected from 354 calves born to these cows were evaluated using a digital refractometer. The minimum, maximum, mean and standard error values were 14.4%, 40.6% and 27.9±0.2%, respectively, for the colostrum Brix and they were 19.7 mg/ml, 168.1 mg/ml and 95.9±1.4 mg/ml, respectively, for colostrum immunoglobulin. The same values were 3.9 g/dL, 10.9 g/dL and 6.5±0.1 g/dL, respectively, for the serum total protein and 2.2 mg/ml, 37.7 mg/ml and 13.6±0.3 mg/ml, respectively, for immunoglobulin. The share of the colostrum samples below Brix 22% was 8.2% and that of the samples with immunoglobulin levels below 50 mg/ml was 3%. The percentage share of calves with a serum total protein level below 5.5 g/dL was found to be 15%, while the percentage of the immunoglobulin levels below 10 mg/ml was found to be approximately 30%. Birth season, lactation number, birth type and body condition score were found to have significant effects on colostrum quality (P<0.05). The serum total protein and immunoglobulin levels of female calves born in spring-summer to cows in their 4th and successive lactation and consuming at least 3 L colostrum Brix 30% in the first feeding were higher than in the others. Colostrum quality of cows and serum characteristics of calves should be evaluated in dairy farms. This is especially important for farms where calf deaths are more prevalent. Because of varying absorption ability, it may be advisable to give Holstein calves 3 L colostrum including IgG with min. Brix 23% or min. 80 mg/ml in the first feeding.

KEYWORDS: colostrum Brix / digital refractometer / immunoglobulin / serum total protein

Milk produced within 24 hours following birth is called colostrum and the one produced within the following 2-3 days is called transition milk [Yang *et al.* 2015].

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Colostrum which differs in composition from normal milk is very important for calves. Colostrum is helpful in the removal of meconium with its laxative effect, while also being the primary source of energy to protect body heat and future performance [Mejer 2015]. However, the most important benefit of colostrum for calves is that, by providing passive transfer (PT), it helps calves acquire immunisation against bacterial diseases that cause deaths in calves. Neonatal calves lack immunoglobulins (Ig) and their placenta is impermeable to Ig. Until their immune system is developed calves can protect themselves against diseases only thanks to these substances in the colostrum [Godden 2008]. For this reason, neonatal calves should consume at least 2 L colostrum within 0.5-1 hour after birth at about 10% of their birth weight within the first 12 hours. The most important reason why colostrum is given immediately after birth is that the ability of neonatal calves to absorb the antibodies is rapidly reduced [Kehoe *et al.* 2007].

The fact that colostrum is denser than normal milk and has a yellowish color indicates that it is rich in Ig. The most important factor determining colostrum quality is the level of gamma immunoglobulin (IgG) [McGuirk and Collins 2004]. From the first milking on, the quality of colostrum deteriorates. Factors affecting colostrum quality include breed, animal, age or lactation number of the cow, month or season of calving, dry period length, dry period care management practices, gender of the calf, health of the cow, vaccination program, milk leak from teat before calving as well as colostrum density and amount [McGuirk and Collins 2004].

Various methods are used to measure colostrum quality, i.e. to determine Ig types and quantities. Of these, radial immunodiffusion (RID) and direct biosensor SPR-immunoassay are the basic laboratory methods. Spectrophotometers, lactodensimeters, colostrometers (hydrometers), optical and digital Brix refractometers are measurement tools developed for practical use in farms [Fleenor and Stott 1980, Doepel and Bartier 2014].

Since there is a high correlation between the RID and Brix refractometer results (+0.64 - +0.94), the use of optical or digital refractometers has been suggested in many studies in order to determine colostrum quality and serum IgG levels [Bielmann *et al.* 2010, Morril and Tyler 2012, Quigley *et al.* 2013, Chigerwe and Hagey 2014, Deelen *et al.* 2014]. There are three basic Igs in the colostrum known as IgA, IgM and IgG (IgG1, IgG2) [Collier *et al.* 2012]. Although both IgA and IgM contribute to passive immunity of the calf, 90% of the total Ig content in colostrum is IgG1 [Godden 2008, Villarroel *et al.* 2013, Verweij *et al.* 2014]. The Ig density reaches its peak level 5-10 days before birth. While IgA and IgM are produced locally within the mammary gland, most of the IgG is derived from the body fluids [Collier *et al.* 2012]. Although at least 50 g/L IgG is assumed for quality colostrum, different Brix threshold values have been reported [Bartier *et al.* 2015, Bartens *et al.* 2015, Bielman *et al.* 2010, Moore *et al.* 2009, Quigley *et al.* 2013, Morril *et al.* 2015].

PT levels of calves are directly determined by considering their serum IgG levels. The use of RID in farms to measure IgG levels may not be appropriate in terms of time, cost and practicality. For this purpose serum TP levels measured with a refractometer, which is an indirect method, may be used [Doepel and Bartier 2014].

It is necessary to give more quality colostrum to calves with a weak immune system and provide them with greater care. Calves consuming higher quality colostrum are equipped with a faster immune mechanism and healthier gut [Yang *et al.* 2015]. Factors affecting the immune system of calves include the dam's age, calf's birth weight, gender as well as the amount and quality of colostrum. If serum contains IgG <5 mg/ml in calves, this is classified as failure of passive transfer (FPT); if it is IgG = 5-10 mg/ml, as partial passive transfer (PPT), if IgG≥10 mg/ml, as adequate passive transfer or immunity (successful, normal, APT, NPT) [Godden, 2008, Conneely *et al.* 2014, Villaroel *et al.* 2013, Doepel and Bartier 2014]. Furthermore, PT threshold values for serum TP levels were reported as FPT <5.2 g/dL, PPT = 5.2-5.5 g/dL and APT≥5.5 g/dL [Godden 2008].

It has been stated that serum IgG 10 mg/ml corresponded to Brix 7.8% [Moore *et al.* 2009]. Neonatal calves need to take and absorb 100-200 g of IgG in order to acquire adequate passive immunity [Doepel and Bartier 2014]. It was reported that the percentage of herds with PT in the USA was 2.1% [Morrill and Tyler 2012], while the rate of FPT in calves was about 20% [Morrill *et al.* 2013, Doepel and Bartier 2014]. In addition, the share of female calves that could not consume enough colostrum within 24 hours after birth was more than 40% [Quigley 2001, Yang *et al.* 2015].

The objective of the present study was to estimate the effects of some factors on colostrum quality in Holstein calves.

Material and methods

Collection and analysis of colostrum and serum samples

In the present study, 332 colostrum samples were taken from 334 Holstein cows, which calved between December 2016 and July 2017 in two herds in Turkey. In addition, serum samples collected from 354 calves of these cows were also analysed. The colostrum samples were taken during the first milking and stored at -20°C until analysis. In the first half hour after calving, 8 kg of colostrum is collected from cows milked with a portable milking machine. Moreover, blood samples of the calves were collected by farm veterinarians from vena jugularis 36 hours after birth and then serum was separated in a centrifuge and finally stored at -20°C. Analyses of colostrum and serum samples were performed using a digital refractometer (Palm Abbe Digital Refractometer # PA203, Misco, Cleveland, Ohio, USA). In the refractometer the colostrum results were read as Brix% and the serum TP results as g/dL. The Brix scale of the digital refractometer used in the study was between 0-56% and the serum TP scale was between 1-14 g/dL, respectively. The Brix value in liquids with no sucrose content provides an approximate solids content. The Brix values read for colostrum were converted to IgG (mg/ml) values using a conversion table as recommended by the manufacturer.

Data analysis

Some corrections were made in the data obtained in the study prior to the statistical analysis. For cows in one of the herds no information was available concerning 305 -day milk yields in previous lactations, dry period length, body condition scores (BCS), incidence of dystocia, colostrum amounts taken and cases of septicemia. In the preliminary analysis it was observed that the herd effect was significant. Therefore, in accordance with the literature review, the effect of the herd factor was eliminated and the sets of data were corrected in terms of this factor [Waldner and Rosengren 2009]. The number of the remaining factors was 12 and the number of the factors known for both herds was 6. In order to determine the effect of the remaining 6 factors, the sets of data belonging to only one herd were used. While 9 fixed factors were added to the model to be used for assessing the colostrum properties, 12 fixed factors were added for the serum properties. These computations were performed using the Minitab package programs [Minitab 2010]. The structures of the models used are shown in Table 1. Some additional arrangements were made for the levels of the factors used for the data analysis. These included the calving season (CS): winter: December, January, February, spring and summer: April, May, June, July; gender: male, female; lactation number (LN): 1, 2, 3, 4+ (4 and the above combined); birth type (BT): single, twin; health problems at birth (HP): yes, no; assistance at birth (AB): yes, no; body condition score (BCS): 2.5-3.0, 3.5, 4.0; 305 day milk yield (305 DMY): low <9000, medium ≤9000 - <10500, high ≥10500 kg; dry period (DP): short ≤70, long >70 days; colostrum group (CG): weak <22, medium ≤ 22-<25, good ≤ 25-<30, very good ≥30; first colostrum consumption (FCC): 2.0, 2.5, 3.0 L; hyperimmune serum injection (HSI): whether 20 mL of serum (Bovi-Sera Serum Antibodies, Colorado Serum Company, USDA Code: 3391.00) were injected subcutaneously.

Table 1. Structures of the statistical models used to evaluate the effects of factors on colostrum and serum properties

Factor	Colostrum properties		Serum properties	
	all data	herd_1	all data	herd_1
Calving season	+	+	+	+
Sex	+	+	+	+
Lactation number	+	+	+	+
Birth type	+	+	+	+
Health problems at birth	+	+	+	+
Colostrum group			+	+
Assistance at birth		+		+
Body condition score		+		+
305 day milk yield		+		
Dry period		+		
First colostrum consumption				+
Hyperimmune serum injection				+

Results and discussion

Colostrum quality

The descriptive statistics of the properties examined in the study are given in Table 2. The mean, minimum and maximum values for Brix were 27.9, 14.4 and 40.6%, respectively. A total of 8.2% samples were under Brix 22% and evaluated as weak, 15.6% as moderate and 76.2% as good and very good (Tab. 3). It was found that the average IgG was 95.9 mg/ml, the percentage of the weak samples containing a IgG level below 50 mg/ml was 3%, the share of those containing an IgG level ≤50 - <80 mg/ml was 20.8% (medium) and the percentage of those containing good and very good quality colostrum was 76.2%.

Table 2. Descriptive statistics for colostrum, serum properties and length of the dry period

Property	N	Mean	SEM	SD	Minimum	Maximum
305DMY (kg)	159	10571	145	1831	4624	15800
DP (day)	236	71	1	15.4	24	167
Colostrum Brix (%)	332	27.9	0.2	4.5	14.4	40.6
Colostrum IgG (mg/ml)	332	95.9	1.4	25.5	19.7	168.1
Serum TP (g/dL)	354	6.5	0.1	1.1	3.9	10.9
Serum IgG (mg/ml)	354	13.6	0.3	6.3	2.2	37.7

305DMY 305 day milk yield; DP dry period, IgG immunoglobulin G, TP total protein, N number of data, SEM standard error of mean, SD standard deviation.

Table 3. Colostrum and serum quality categories and their distributions

Colostrum Brix% group	N	%	Class	Colostrum IgG group (mg/ml)	N	%	Class
<22	27	8.2	weak	<50	10	3.0	weak
≤22-<25	52	15.6	moderate	≤50-<80	69	20.8	moderate
≤25-<30	166	50.0	good	≤80-<120	194	58.4	good
≥30	87	26.2	very good	≥120	59	17.8	very good
Serum TP group (g/dL)	**N**	**%**	**Class**	**Serum IgG group (mg/ml)**	**N**	**%**	**Class**
<5.2	30	8.5	FPT	<5	21	5.9	FPT
≤5.2-<5.5	23	6.5	PPT	≤5-<10	85	24.0	PPT
≤5.5-<6.0	59	16.7	APT	≤10-<15	111	31.4	APT
≤6.0-<7.0	139	39.2	APT	≤15-<20	85	24.0	APT
≥7.0	103	29.1	APT	≥20	52	14.7	APT

N – number of data, FPT – failure of passive transfer, APT – adequate passive transfer, PPT – partial passive transfer.

The factors affecting colostrum quality properties and the least squares means (LSM) calculated for their levels and the standard errors are given in Table 4. Although the effects of the calving season, lactation number, birth type and BCS, i.e. some of the factors affecting colostrum Brix value, were found significant ($P<0.05$), the gender of the calf, health problems of the cow at birth, dystocia, previous lactation 305DMY and the duration of the dry period were found to be non-significant ($P<0.05$). Nevertheless,

Table 4. Least squares means and standard errors for factors affecting colostrum Brix%, colostrum IgG, serum TP and serum IgG levels

Factor	Level	N	Colostrum Brix% LSM±SEM	Colostrum IgG (mg/ml) LSM±SEM	Serum IgG (mg/ml) LSM±SEM	Serum TP (g/dL) LSM±SEM
Calving season	winter	187	28.6±0.5^{b*}	100.3±2.6^{b*}	11.8±0.7^{b**}	6.1±0.1^{b**}
	spring-summer	142	29.7±0.5^{a}	106.4±2.9^{a}	13.8±0.7^{a}	6.5±0.1^{a}
Sex	male	143	29.3±0.5	104.3±2.8	11.9±0.7^{b**}	6.2±0.1^{b*}
	female	186	28.9±0.5	102.3±2.7	13.7±0.7^{a}	6.5±0.1^{a}
Lactation Number	1	106	28.9±0.6^{ab*}	102.2±3.2^{ab*}	11.7±0.8^{b*}	6.1±0.1^{b*}
	2	78	28.1±0.6^{b}	97.3±3.4^{b}	12.8±0.8^{ab}	6.4±0.1^{ab}
	3	61	29.9±0.6^{a}	107.8±3.5^{a}	13.0±0.9^{ab}	6.4±0.2^{ab}
	4+	84	29.6±0.6^{a}	106.0±3.3^{a}	13.8±0.8^{a}	6.5±0.1^{a}
Birth type	single	298	27.1±0.3^{b*}	99.6±1.5	12.8±0.4	6.3±0.1
	twin	31	29.1±0.8^{a}	107.0±4.4	12.8±1.1	6.3±0.2
Health problems at birth	yes	179	29.1±0.5	102.9±2.6	12.6±0.7	6.3±0.1
	no	150	29.2±0.5	103.8±2.8	13.0±0.7	6.4±0.1
Colostrum Brix Group	<22	27			9.5±1.3^{c**}	5.7±0.3^{c**}
	≤22-<25	52			11.4±0.9^{bc}	6.3±0.2^{bc}
	≤25-<30	165			13.7±0.6^{b}	6.5±0.2^{b}
	≥30	85			16.6±0.8^{a}	7.0±0.2^{a}
Assistance at birth	yes	23	30.5±1.7	142.3±18.5	11.7±1.4	6.2±0.2
	no	96	29.2±0.9	125.8±10.4	13.1±0.9	6.5±0.1
BCS	2.5-3.0	51	26.7±1.0^{b*}	132.2±12.2	12.3±1.2	6.3±0.2
	3.5	36	29.9±1.5^{a}	141.6±18.9	12.9±1.4	6.5±0.3
	4.0	32	29.1±1.2^{ab}	128.2±16.1	12.0±1.5	6.3±0.3
305DMY	<9000	16	30.9±1.5	145.5±16.3		
	≤9000<10500	35	29.4±1.4	129.9±14.8		
	≥10500	28	29.2±1.5	126.8±15.8		
DP	≤70	44	30.2±1.4	138.8±15.5		
	>70	35	29.4±1.2	129.3±12.5		
First colostrum consumption (L)	2	21			10.5±1.4^{b**}	6.1±0.2^{b*}
	2.5	73			11.9±1.1^{b}	6.3±0.2^{b}
	3	25			14.6±1.4^{a}	6.8±0.3^{a}
Hyperimmune serum injection	yes	50			9.8±1.6^{b**}	5.9±0.3^{b**}
	no	28			13.8±1.7^{a}	6.6±0.3^{a}

N number of data, LSM Least square means, SEM standard error of mean.
**P<0.01, *P<0.05.
$^{ab\cdots}$Within columns the means bearing different superscripts differ significantly at P<0.05.

only the effects of the calving season and lactation number on the colostrum IgG level were found significant (P<0.05). Higher Brix% and IgG levels were recorded in colostrum of cows that calved in the spring-summer season in comparison to those calving in the winter.

Although colostrum quality of cows in their 3rd and 4th+ lactations was found to be higher, there was no statistically significant difference between them and those in the 1st lactation (P>0.05). Nevertheless, the lowest quality colostrum was obtained from cows in the 2nd lactation. Although cows giving birth to twin calves compared to those with single calves, cows with a dry period below 70 days vs. those with longer dry periods, cows with dystocia vs. those with an easy birth, cows with a BCS of 3.5 compared to the others and cows with milk yields of below 9000 kg in the previous lactation compared to those with greater milk yields had higher colostrum IgG contents, the differences were statistically non-significant (P>0.05).

When Brix 22% and IgG 50 mg/mL are accepted as the threshold values, it can be stated that colostrum quality of cows included in this study was relatively good. In some studies colostrum quality in Holstein cattle was determined using a colostrometer, with 10% and 12% of the samples reported to be of low quality [Hoyraz *et al.* 2015, Kaygısız and Köse 2007, Göncü *et al.* 2013]. However, in other studies the percentage of low quality colostrum was higher than the value found in the present study and the mean Brix values were lower [Quigley *et al.* 2013, Chigerwe and Hagey 2014, Doepel and Bartier 2014, Mejer 2015].

In this study the lowest colostrum IgG level was recorded in cows in their second lactation. Similarly to the results of the present study, it was reported that colostrum of cows in the 3rd+ lactation contained more IgG than that of cows in the 1st and 2nd lactations [Doepel and Bartier 2014]. It is stated that since young cows are not exposed to diseases as frequently as old cows and have fewer protective antibodies, they might have lower IgG levels in their colostrum [Waldner and Rosengren 2009]. On the other hand, Mulder *et al.* [2017] found that while older cows produced lower quality colostrum due to the decline in BCS in the dry period as a result of weight loss, cows in the 1st lactation produced higher quality colostrum.

In the present study it was established that assistance at birth had no statistically significant effect on the colostrum Brix and IgG, although it increased the IgG level. Although it was statistically non-significant, greater IgG contents were found in the colostrum of cows with 3.5 BCS when compared to the others. Colostrum of cows with 305-day milk yield below 9000 kg in the previous lactation and a dry period shorter than 70 days had higher Brix and IgG levels. On the other hand, it was reported that the effects of the dam's age, calving season, birth type, gender and dystocia on the colostrum IgG level are non-significant in Sarabi cattle [Rezazadeh *et al.* 2016].

Serum TP and IgG levels

Mean serum TP and IgG values were 6.5 g/dL and 13.6 mg/ml, respectively. The minimum and maximum values were 3.9, 10.9 g/dL for TP and 2.2, 37.7 mg/ml for IgG (Tab. 2). It was found that the percentage of calves with serum TP levels below 5.2 g/dL was 8.5% and the share of calves with IgG levels below 5 mg/ml was 5.9%, thus that these calves were exposed to FPT. The calves that had IgG levels between ≤5 and <10 mg/ml had acquired PPT and they accounted for 24.0% of the total number of calves analysed in this study (Tab. 3). It was found that 70.1% calves had IgG levels of min. 10 mg/ml and provided APT.

When considering the amount of colostrum initially consumed by the calves and the colostrum IgG level of each sample, the amount of IgG consumed by each calf was determined in only one herd. While the initial average IgG consumption was calculated as 234.1 g/L, the percentage of calves with IgG consumption below 100, 150 and 200 g/L was 3.8, 13.2 and 34.0%, respectively.

The effects of calving season, gender, lactation number, colostrum Brix group, amount of colostrum consumed and hyperimmune serum application on TP and IgG were statistically significant ($P < 0.05$, Tab. 4). Female calves born in the spring and summer from the older cows and consuming better quality colostrum in the amount of 3 L were found to have higher serum TP and IgG levels compared to the other calves. Furthermore, it was also determined that calves not administered hyperimmune serum had 4 mg/ml more IgG. The effects of the other factors on serum TP and IgG levels were found to be statistically non-significant ($P>0.05$).

The serum TP and IgG means were found to be higher in the present study compared to the values reported in some studies [Perino *et al.* 1993, Villarroel *et al.* 2013, Doepel and Bartier 2014] and they were comparable to those found in some others [Deelen *et al.* 2014, Yang *et al.* 2015]. Although the percentage of calves consuming less than 100 g/L of colostrum containing insufficient IgG in the first feeding was below 5%, 15% calves in terms of serum TP and approximately 30% calves in terms of IgG displayed FPT and PPT at the 36th hour. This demonstrates that although some calves consumed sufficient IgG levels, they were exposed to FPT, which indicated that their absorption activities were insufficient.

While the IgG level in calves of cows with colostrum below Brix 22% was 9.5 mg/ml, it was 16.6 mg/ml in calves of cows with min. Brix 30%. Similarly, serum IgG levels of calves consuming 3 L colostrum were higher than in calves consuming 2 L and 2.5 L colostrum. Although it is known that, as a standard, at least 2 L high-quality colostrum should be given within the first 6 hours in order to obtain a successful passive Ig transfer (10 mg/ml), it was reported that calves given 3 L colostrum in the first feeding had more successful PT [Osaka *et al.* 2014]. However, of calves consuming colostrum at as much as 7%, 8.5% and 10% their body weights, the highest IgG level was found in the serum of calves consuming colostrum as much as 8.5% of their live weight [Conneely *et al.* 2014]. The researchers reported that this might have occurred due to the higher absorption activity of these calves.

It was observed that calves born in the spring and summer months are characterised by more successful PT than those born in the colder months. It was found that female calves had higher levels of serum TP and IgG than their male counterparts ($P<0.01$). According to these results, it can be concluded that female calves had more effective absorption and, as a result, were characterised by more successful PT. These results are compatible with some research findings [Vogels *et al.* 2013, Mulder *et al.* 2017].

Although colostrum of the twin-calving cows had a lower Brix value, no difference was found between the serum TP and IgG levels of the single and twin born calves. This may be explained by the higher absorption capacity of twin-born calves in comparison to single-born calves even though the twin calves received lower quality colostrum. Similarly to the findings of the present study, it was stated that calves that received help at birth compared to normal-born calves and calves born from cows with bad BCS at birth compared to the ones with good BCS had lower IgG levels [Selk 2018]. Nevertheless, Furman-Fratczak et al. [2011] reported that the main factors causing FPT and PPT were dystocia and poor survival power resulting from insufficient colostrum consumption.

Hyperimmune serum injection had a negative effect on PT of calves. These results are comparable to those obtained by Selim *et al*. [1995]. Similarly, it was reported that intravenous 0.5 L hyperimmune serum transfusions increased serum IgG levels in neonatal calves with FPT, but this increase was not sufficient to provide APT [Chigerwe and Tyler, 2010]. However, it is reported that the administration of appropriately prepared hyperimmune plasma was recommended for protection and treatment of neonatal calf diarrhea and might reduce the use of antibiotics [Yüceer and Özbeyaz 2010, Bresciani *et al.* 2016].

In a study conducted on Angus cattle it was found that calf gender did not affect the serum IgG level and twin born calves compared to single born and those born in the case of dystocia compared to the ones with normal birth had lower IgG levels [Waldner and Rosengren 2009]. Researchers reported that dystocia could cause injury, stress or fatigue for both cows or calves and this could negatively affect IgG absorption.

As a result, in modern commercial dairy herds both colostrum quality and PT levels of calves should be determined. For this purpose a practical, quick, cheap optical or digital refractometer can be used. Breeders can make more accurate maintenance management decisions based on testing results. In the herds where calf mortality rates are above the acceptable value limits, higher quality colostrum should be used due to the different absorption abilities of calves. It may be necessary to set a higher threshold value, especially to compensate for potential errors made while determining colostrum quality. For this reason it can be suggested that calves should be given colostrum with Brix 23% or higher or IgG level of 80 mg/ml within the first 24 hours. It can be stated that it will be more appropriate to give normal Holstein calves 3 liters of colostrum within the first half hour for successful PT, thus leading to a reduction in the risk of mortality.

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